PREDICTING TADPOLE METAMORPHOSIS: ECOLOGICAL ASPECTS OF ENERGY ALLOCATION AND DEVELOPMENT

BY

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Amphibian larvae exhibit plasticity in age and size at metamorphosis. Ecological models of amphibian metamorphosis might be improved by considering how tadpoles allocate energy to storage versus growth or development.

I experimentally manipulated food availability to test whether fat storage in tadpoles is independent of body size. Fat reserves at metamorphosis were correlated with body mass, but size-adjusted reserves varied significantly among feeding treatments. Allocation to lipid storage is therefore plastic, and warrants consideration in metamorphosis models.

In a second experiment I examined the relationship between growth and development in Bufo terrestris tadpoles in light of a model of metamorphosis based on changing

priorities of allocation to growth versus development. Developmental trajectories indicated that plasticity in development rate persists through later stages than indicated by previous studies. Tadpoles can delay metamorphosis and take advantage of opportunities for rapid growth. In contrast to previous studies, loss of plasticity in age at metamorphosis appeared to be the product of time constraints on development rather than an actual loss of flexible development rate. The data support the model of dynamic allocation.

The same experiment was also used to test whether lipid storage is significantly related to developmental timing, and how lipid storage relates to a model of proportional energy allocation in tadpoles. Timing of metamorphosis was positively correlated with higher size-adjusted lipid storage. I propose an expanded model of proportional allocation that relates lipid storage to rapid development. Allometric effects generally dominate allocation to storage, but stage-specific effects dominate under some conditions.

Tadpoles facultatively adjust development rate in unpredictable habitats. I predicted that lipid storage would be reduced when environmental conditions induced accelerated development. This prediction was not supported in a comparison of tadpoles from artificial ponds that dried early versus constant-depth ponds. Mean lipid storage did not differ between drying versus constant ponds, but tadpoles in drying ponds stored lipid faster per unit body size. This

result is consistent with the proposed model of stage-specific allocation to storage. Further research on the ecology and genetics of phenotypic plasticity in complex life cycles will help characterize the adaptive significance of stage-specific allocation patterns.

CHAPTER 1
INTRODUCTION

Organisms live in a wide variety of environments that range from very stable to those that fluctuate dramatically and unpredictably. For example, communities around hydrothermal vents in the ocean floor are among the most stable, with constant temperature, no change of seasons, and constant energy input. In contrast, many environments such as tree-fall gaps in rainforest canopies or ephemeral rain puddles in deserts, may be highly unpredictable in space or time. Nonetheless, many organisms rely on such environments. How organisms deal with environmental unpredictability is a central theme in ecological research.

One of the defining characteristics of living organisms is the ability to assimilate energy from the environment (Purves and Orians 1983, Wessells and Hopson 1988). Energy availability is one factor that often varies unpredictably in both terrestrial and aquatic environments. Assimilated energy must be allocated among the competing functions of maintenance, growth, reproduction, or activity, including the acquisition of more energy (Sibly and Calow 1986, Boggs and Ross 1993). Assimilated energy may be allocated immediately, or stored for future allocation. Because patterns of energy allocation will affect an organism's survival and fecundity,

energy allocation is a fundamental component of life histories (Stearns 1992, Perrin and Sibly 1993). Life history research is often focused on characterizing how a species' life history is suited to its environment, and how life histories change in response to environmental variation.

Perrin and Sibly (1993) provided a thorough review of current models of energy allocation, including models for both constant and unpredictable environments. These models begin with the assumption that selection will tend to optimize energy allocation, thereby maximizing fitness. In these models energy storage is considered adaptive under certain conditions, depending on the relative benefits of immediate versus delayed allocation. Unpredictability in the returns from somatic and reproductive allocation may select for energy storage (Perrin and Sibly 1993). Thus in unpredictable environments, energy storage is likely to be adaptive.

This question of how organisms deal with unpredictable environments becomes more complex when we consider species that exploit two or more distinct environments over the course of a complex life cycle. Wilbur (1980) defines a complex life cycle as one in which there is a larval stage that undergoes a radical morphological metamorphosis accompanied by a change in habitat. Sexual maturity is an important life history transition for many organisms, and its timing often varies in response to environmental variables. Similarly, metamorphosis of species with complex life cycles

is a phenotypically plastic trait that is influenced by many environmental factors (Wilbur and Collins 1973, Wilbur 1980). While metamorphosis and sexual maturity often occur together, this is not always the case in complex life cycles. For example, in amphibians metamorphosis may occur long before or, in some cases, after sexual maturity, and may impose independent selective pressures on energy allocation.

Amphibians represent an excellent system for studying how animals with complex life cycles allocate energy in response to unpredictable environmental variation. Typically amphibians have aquatic larvae that metamorphose into terrestrial juveniles. Salamander larvae are carnivorous, as are the adults, and sexual maturity in salamanders may occur well before metamorphosis or years afterward. In contrast, most anuran larvae are suspension-feeding herbivores or omnivores that metamorphose into carnivorous adults. For frogs metamorphosis is a prerequisite for sexual maturity. Thus metamorphosis in frogs marks a radical life history transition, a change in morphology, diet, and habitat, that is essential for individual reproductive success.

In anurans variation in age and size at metamorphosis has been shown to have fitness consequences. Age and size at metamorphosis are correlated with survival to maturity (Berven 1990) and age at first reproduction (Collins 1979, Smith 1987, Berven 1990). Larger size at metamorphosis may reduce the risks of mortality from predation, desiccation, and starvation in the terrestrial habitat (Smith 1987). In

addition, size differences at metamorphosis can persist to maturity (Smith 1987, Berven 1990) and thus affect male mating success and female fecundity (Howard 1978, Berven 1990).

Anurans typically inhabit unpredictable environments as larvae, and metamorphosis is an obligate life-history transition with significant fitness consequences. So how do tadpoles accommodate environmental variation? One factor that must be considered is the importance of growth. Wassersug (1975) has argued that the tadpole stage of the anuran life cycle is adapted to exploit bursts of primary productivity that occur when temporary ponds fill, and that growth is the primary function in the tadpole phase. The importance of tadpole growth in determining age and size at metamorphosis is seen in ecological models for predicting metamorphosis.

Wilbur and Collins (1973) proposed a model for predicting age and size at metamorphosis which has become central to our understanding of phenotypic plasticity in amphibians. According to this model, the various environmental factors that influence larval development exert their influence by affecting tadpole growth rates. There is a minimum body size (b) that must be attained before tadpoles are physiologically competent to metamorphose. A tadpole's growth rate determines how rapidly it reaches b, and thus how soon metamorphosis is possible. Once tadpoles attain this minimum size, however, they may metamorphose immediately or

delay metamorphosis and take advantage of opportunities for further growth. The "decision" to metamorphose or not is also dependent on growth rate. According to the Wilbur-Collins model, as long as a tadpole's growth rate remains relatively high, metamorphosis is delayed. The model proposes a minimum threshold of mass-specific growth rate (g) above which tadpoles delay metamorphosis; if mass-specific growth drops below g, metamorphosis is initiated.

The Wilbur-Collins model allows predictions of how environmental variation will influence age and size at metamorphosis. Consider two groups of tadpoles in different environments, where differences in habitat impose different limits on growth rate. The slowly-growing group will reach b later than tadpoles in a rapid-growth environment, and once they reach b, their growth rates will fall below g more rapidly. Thus they will metamorphose later, and smaller than tadpoles in the high-growth environment.

In addition to comparing tadpoles with different mean growth rates, the model can also be used to predict the effects of changes in growth rate at different times during the larval period. For example, a decline in growth rate prior to attainment of b will delay metamorphosis relative to tadpoles that do not experience the decline. In contrast, a decline in growth rate after attainment of b will accelerate metamorphosis. An early increase in growth rate will allow tadpoles to reach b sooner and will result in earlier metamorphosis. An increase in growth rate after b is

reached may result in either accelerated or delay metamorphosis, depending on the exact values of b, g, and the magnitude of the change in growth rate. These predictions are presented graphically in Alford and Harris (1988) and Hensley (1993).

This model of amphibian metamorphosis has proven to be a robust paradigm for understanding plasticity in age and size at metamorphosis. It emphasizes the importance of growth rate in determining age and size at metamorphosis, which are factors that can significantly affect individual fitness. Growth, however, is but one function that competes for energy assimilated from the environment. In unpredictable environments energy storage may be an important, adaptive allocation priority. Given that there is clearly a relationship among growth, development, and energy storage, how should a tadpole allocate energy in an unpredictable environment?

I conducted three experiments to address the question of how tadpoles allocate energy in unpredictable environments. First, I examined the assumption that energy allocation in tadpoles is phenotypically plastic. Most of the research on plasticity in tadpole growth and development has emphasized the importance of body size and growth rate. I tested whether tadpole fat storage varies with environmental conditions (changes in food supply) independently of body size. Second, I tested two recent models of amphibian plasticity (Leips and Travis 1994, Harris *in press*) that

emphasize energy allocation rather than growth. These models make specific predictions about changes in age and size at metamorphosis in response to environmental variation. Again, I used tadpole responses to changes in food availability to test predictions of the models. Finally, I performed an experiment to test how fat storage is affected by unpredictable pond duration. Previous work has demonstrated that tadpoles can facultatively adjust development rates in response to early pond drying. I tested whether fat storage is affected by early pond drying as predicted by models of energy allocation in tadpoles.

CHAPTER 2
ENERGY ALLOCATION, GROWTH, AND DEVELOPMENT: PLASTICITY IN LIPID STORAGE IN TADPOLES

Introduction

Amphibian larvae exhibit phenotypic plasticity in growth and development rates, and thus a great deal of variation in age and size at metamorphosis. This plasticity has received much attention in experimental ecology (reviews in Alford *in press*, Harris *in press*). This research has been motivated by a desire to understand how these phenotypes respond to environmental variation, and whether such responses are adaptive (review in Newman 1992). Several models have been proposed to predict age and size at metamorphosis (reviews in Alford 1988, Hensley 1993, Harris *in press*). In these models individual growth rate is a critical determinant of age and size at metamorphosis.

The most widely applied model (Wilbur and Collins 1973) proposes that there are minimum and maximum body size limits on metamorphosis. Growth rate, which is determined by various factors in the larval environment, influences how rapidly tadpoles reach the minimum size for metamorphosis and their tendency to accelerate or delay metamorphosis once the threshold size has been reached. Growth rates also play an important role in alternative models proposed by Werner

(1986) and Rowe and Ludwig (1991) that focus on population optima of age and size at metamorphosis rather than simply individual responses.

Although experimental work has been conducted to test various aspects of the models (reviews in Alford 1988, Hensley 1993, Harris *in press*), one factor that has not been studied in detail is energy allocation. All organisms must allocate assimilated energy among the competing functions of growth, maintenance, reproduction, and storage (Sibly and Calow 1986). Wassersug (1975) has argued that the tadpole stage is an adaptation for exploiting transient opportunities for rapid growth. Given that growth is a high priority for tadpoles and is predictive of age and size at metamorphosis, tadpoles might be expected to allocate energy accordingly.

Crump (1981) studied energy accumulation in tadpoles of the spring peeper Pseudacris (Hyla) crucifer. In her study tadpoles raised at low densities accumulated more total energy and more energy per unit mass than did tadpoles raised at high densities. Based on these results, Crump proposed that energy accumulation may be an important determinant of age and size at metamorphosis, and that metamorphosis may not be possible without a minimum amount of stored fat.

Crump's study raises the basic question of how tadpoles allocate assimilated energy between, for example, increasing body size, and increasing energy density through fat storage. One possibility is that energy allocation is strictly allometric; animals follow a fixed pattern of increasing

allocation to storage as body size increases. Such a fixed allocation rule would explain Crump's observation of higher energy density in larger tadpoles, but phenotypic plasticity in body size would adequately account for the differences in energy content.

Alternatively, energy allocation may be a phenotypically plastic trait that is part of the complex metamorphic phenotype. If energy allocation is strictly allometric, then it would be unnecessary to consider energy accumulation as a possible size-independent determinant of metamorphosis. If, on the other hand, energy allocation is a phenotypically plastic trait independent of body size, this plasticity would support Crump's proposal that energy accumulation is a factor that should be considered in predicting metamorphosis. Further, plasticity in allocation would suggest that energy reserves at metamorphosis might influence fitness independently of body size.

I tested whether energy allocation is phenotypically plastic in Pseudacris crucifer tadpoles, and examined how tadpoles allocate assimilated energy between growth and storage in response to changing food availability. Because genetic variation for age and size at metamorphosis has been demonstrated in several previous studies (Travis 1980, Newman 1988a, Semlitsch et al. 1990), I measured the responses of four separate full-sibling families in this study. Variation among sibships in growth and development may be genetic, or may be due to non-genetic maternal effects such as variation

in egg size or composition. Travis (1980) pointed out that if growth is size-specific, initial hatchling sizes may have strong effects on growth rates, and ultimately influence metamorphic phenotypes. Kaplan (1992) found that egg size interacted with temperature, significantly influencing embryo development rate and hatchling survival. I therefore measured egg size (diameter) and hatchling size (body length) for all tadpoles used in this study.

Methods

I raised tadpoles individually from hatching to metamorphosis on controlled food rations. For each tadpole, age and size at metamorphosis were recorded, and lipid storage was measured using petroleum ether extraction.

Feeding treatments were either constant high food (H), constant low food (L), or a switch (low increased to high (I) or high decreased to low (D)). The H and L groups are considered control groups. Each treatment-sibship combination was replicated twice on each of 8 laboratory shelves that were treated as spatial blocks. Food switches (I,D) were made after 14 days because previous work (Hensley 1993) indicated this would generate significant effects on age and size at metamorphosis compared to unmanipulated controls.

Pseudacris crucifer (Hylidae) is a winter-breeding treefrog that lays its eggs in temporary ponds. I collected

11 amplectant pairs from a Carolina bay, a natural temporary wetland, at the intersection of roads 2 and F on the Savannah River Site in Aiken County, South Carolina, on 24 January 1993. Pairs were placed individually in plastic containers with water and vegetation from the bay and allowed to lay eggs. The next morning four clutches of eggs were randomly selected for the experiment. I haphazardly selected 64 eggs from each of the four clutches, separated them using fine forceps, and placed each egg in a polyethylene cup (9.5 cm tall x 9.2 cm diameter) containing 38 ml of well water. Eggs were randomly assigned to one of the four feeding treatments. Egg diameters were measured under a dissecting microscope using a video camera and MorphoSys® image analysis software. For each egg I measured two approximately perpendicular diameters and calculated a mean. During the four hours required to measure the eggs no trend of change in egg size within clutch or shape (elliptical eccentricity) was detected. Additional eggs from each of the four clutches were also analyzed for total lipid content and various lipid classes (Komoroski, *unpublished data*).

I measured snout-vent length of the four females whose eggs were chosen for the experiment, and counted the total number of eggs laid by each female. Females were killed by anaesthetic overdose using MS-222, preserved in formalin, and dissected to allow counts of unlaid eggs.

Eggs hatched in 3 or 4 days. Six days after oviposition, I measured total length (snout to tail tip) of

all hatchlings, using the video image analysis system. After measuring lengths, I increased the volume of water in the cups to 370 ml (7.7 cm deep), and replaced all dead tadpoles with similarly-treated siblings (N = 18). Tadpoles were first fed seven days after oviposition, when all tadpoles were at stage 25 (Gosner 1960).

Food was delivered using a glass jar with a perforated metal lid. Each shake of the jar delivered 13.3 mg (c.v. = 14.58%) of food. The diet consisted of a finely ground mixture (1:1 by mass) of Purina® rabbit chow and NutraFin® fish flakes. Tadpoles were either fed a high food level (2 shakes) or a low food level (1 shake). Tadpoles were fed on days 7,10,14,18, and every third day thereafter. I changed water prior to each feeding. Based on previous experience, I anticipated that temperature differences among shelves would exist and would contribute to differences among blocks. To check for such a trend, I measured water temperature in a cup near the center of each shelf on 11 haphazardly chosen days during the experiment.

For each tadpole I recorded the day of emergence of at least one forelimb (stage 42, Gosner 1960), and the day when tail resorption was complete (Gosner stage 46). Size at metamorphosis was measured as total dry mass at stage 46.

Lipid reserves of each individual were estimated using methods modified from Reznick and Braun (1987). Whole frozen metamorphs were dried in thrice tared, $^1/_2$ dram glass shell vials at 55°C. Dried tadpoles were stored over $CaSO_4$

desiccant and weighed three times to the nearest 0.1 mg. Lipids were extracted by soaking each tadpole in room-temperature petroleum ether, which preferentially dissolves non-polar storage lipids (triglycerides and fatty acids) (D.L Schultz, *personal communication*; Hensley, *unpublished*). At hourly intervals ether was pipetted off and replaced. Previous work indicated that seven one-hour soaks was in excess of that needed to extract the tadpoles to a constant mass (Hensley, *unpublished data*). After seven extractions, tadpoles were again oven-dried, stored over desiccant, and weighed three times. Mean values of tare mass and mass before and after extraction were used to calculate tadpole total dry mass and total grams of lipid extracted. I used this technique to extract lipids from a pooled subsample of eggs (N = 50 - 80) from each female.

Statistical Analysis

Hypothesis tests for the effects of treatment and sibship on age, size, and lipid storage at metamorphosis were performed using multivariate analyses of variance (MANOVA) with SuperANOVA® 1.1 software (Abacus Concepts Inc. 1989). Dependent variables were transformed to meet the assumptions of analysis of variance; total dry mass (g) and days to metamorphosis were $\log_{10}(x+1)$ transformed, and percent lipid was arcsine(square-root(x)) transformed. Egg diameter was significantly correlated with hatchling body length (R =

0.62) and body length had a higher variance attributable to measurement error, so I used egg diameter rather than hatchling body length as a covariate in the analysis. Because egg diameters were significantly different among sibships ($F_{3,248}$ = 600.2, $P < 0.001$, Table 2-1), egg size effects could not be treated as independent of other family effects (genetic or non-genetic maternal effects such as egg lipid content). Thus egg diameter was treated as a covariate nested within families rather than across families. I explored the underlying structure of the MANOVA using univariate ANOVAs and made *post hoc* comparisons among families and treatments using Scheffe's test.

To test whether lipid storage was allometric or plastic, I used a univariate general linear model with Type I sums of squares. This approach tests hypotheses sequentially and thus allows for an ordered, biological interpretation of the data. In this analysis I tested for treatment effects on allocation to lipid storage after first removing genetic background (sibship effects) and allometric effects (dry mass and dry mass^2). I included the quadratic term because fixed allometric allocation might not necessarily be a linear function. This analysis allowed me to test for plasticity in allocation independent of plasticity in body size. In this model size at metamorphosis was entered untransformed as a covariate; the dependent variable (% lipid) was transformed as before.

To quantify plasticity in responses, I calculated the grand mean and its standard deviation for each response within each family. Plasticity is measured as the number of standard deviations that separate the most extreme treatment means (Leips and Travis 1994).

Results

A total of 193 tadpoles metamorphosed, with 10 to 14 tadpoles in each treatment-sibship category. Treatment means of length of the larval period, dry mass at metamorphosis, and lipid percentages are shown in Figure 2-1. In the initial MANOVA, variation in egg diameter within sibships did not explain a significant amount of variation in the dependent variables. Because egg size was confounded with sibship effects and did not explain significant variance within sibships, it was pooled with sibship for the final analysis.

Feeding treatments, sibships, and laboratory shelves had significant effects on the multivariate response vector (Table 2-2). Feeding treatments significantly affected size at metamorphosis and percent lipid, but not development rate (Figure 2-1). The food increase treatment (I) converged with the high food group (H) in size and lipid content, metamorphosing larger and with more fat than tadpoles that remained on low food (L). Likewise, the food decrease

treatment (D) converged with group (L) in size and lipid content, but differed significantly from group H.

The responses of each sibship are plotted separately in Figure 2-2. Sibship significantly affected length of the larval period and percent lipid. Scheffe's tests among sibships indicated that sibship 1 metamorphosed with significantly less stored fat and significantly later than the other families. There were no significant sibship-by-treatment interactions in the experiment, indicating that all four families responded similarly to the treatments. Qualitatively, however, families differed in whether food manipulations (I and D) resulted in trends toward accelerating or delaying metamorphosis as compared to control groups.

The significant block effect on development rate was attributable to a consistent thermal gradient from the top shelf to the bottom. Temperature variance among the shelves changed over the course of the experiment, but any given shelf was almost always warmer than lower shelves and cooler than higher shelves throughout the experiment (Figure 2-3). Orthogonal polynomial contrasts indicated significant linear and quadratic trends for lower (cooler) shelves to result in slower development.

A significant shelf-by-treatment interaction for size at metamorphosis was detected, but the interaction of shelf-by-family was not significant. Overall, the MANOVA model explained 71% of the variance in development rate, 58% of the

variance in dry mass, and 53% of the variance in lipid storage.

The ANCOVA using Type I sums of squares (Table 2-3) indicated significant effects of genetic background (sibship) and body size (dry mass) on allocation to lipid. After accounting for these variables, treatment effects explained a significant portion of the remaining variation.

Measures of plasticity for each family are shown in Table 2-4.

Discussion

The results of this study indicate that energy allocation between growth and fat storage is a phenotypically plastic trait that responds to conditions in the larval environment independently of changes in body size (Table 2-3). In a laboratory experiment Pfennig (1992) found that higher post-metamorphic survival of Scaphiopus multiplicatus was associated with larger fat bodies at metamorphosis. Thus, like age and size at metamorphosis, lipid reserve at metamorphosis is another phenotypically plastic trait that may significantly influence fitness. Such plastic allocation may be important for predicting metamorphosis, and may be the result of optimized allocation that maximizes cost-benefit ratios of competing functions.

Predicting Metamorphosis

Crump (1981) proposed that a prerequisite for successful metamorphosis might be not only a minimum body size, but also a minimum fat reserve, and that models for predicting metamorphosis timing might be improved by including energy accumulation as a predictive variable. Experiments conducted to test models of amphibian metamorphosis have used mass or linear dimensions as measures of growth. The data presented here support the idea that energy accumulation is, to a degree, independent of body mass and influenced by environmental variables. If energy density or fat reserves are independent of size (mass or length), perhaps a better measure of growth would be total energy accumulated (Crump 1981, Ludwig and Rowe 1990).

Using such a measure might improve how well changes in growth rate predict changes in the timing of development. In this study, however, feeding treatments resulted in no significant changes in timing of metamorphosis, unlike previous studies (Travis 1980, Alford and Harris 1988, Hensley 1993); mass at metamorphosis and lipid storage are positively correlated, but not predictive of developmental timing. Under conditions where size at metamorphosis and developmental timing are correlated (either positively or negatively), consideration of energy content or fat reserves

might improve how well timing of metamorphosis can be predicted.

Optimal Energy Allocation

Optimality models are widely used in the study of plasticity of life histories (Roff 1992). Based on expectations of optimality, and if rapid development is advantageous, tadpoles would be predicted to optimize energy allocation such that they would arrive at the minimum size for metamorphosis with the requisite fat reserve (allometric allocation), and only in subsequent growth would allocation be plastic. Once the minimum threshold for metamorphosis is reached, tadpoles would be expected to allocate energy between growth and storage to maximize post-metamorphic survival and thus maximize fitness.

An alternative to such an optimality approach to allocation is a consideration of constraints. If maximizing growth rates is central to the adaptive significance of the tadpole stage (Wassersug 1975), then why do tadpoles ever allocate energy to storage rather than to growth? Perhaps there are other factors such as nutritional availability of minerals that limit growth, and energy can be assimilated faster than required for maximal growth rate. For example, cannibalistic diets can confer growth advantages in tadpoles when compared to very similar diets that provide as much energy but no conspecific tissue (Crump 1990). This result

suggests that rather than energy, some nutrient limits growth, and may be most readily available when conspecifics are eaten. Under conditions where growth is nutrient-limited, one might predict that over time a tadpole's energy density would increase as the surplus of assimilated energy outpaced growth. Studies of the nutritional ecology of tadpoles are necessary to determine if growth is limited by some nutrient rather than by energy accumulation, and thus results in higher lipid storage. Such a system could produce plastic allocation phenotypes across environments that vary in food availability or quality. Thus, detecting apparent plasticity in lipid storage does not necessarily indicate that tadpoles optimize allocation on the basis of fitness costs and benefits. If development is nutrient-limited, energy allocation may have no influence on development rate.

Costs of Plasticity

Cost of phenotypic plasticity is an important consideration in understanding the evolution of reaction norms. Newman (1992) discussed the difficulty of distinguishing between costs of plasticity across environments and costs of individual phenotypes. Costs of plasticity are incurred due to trade-offs between plasticity in one trait and either the mean contribution to fitness of another trait, or plasticity in another trait. Any single phenotype may have associated costs that are always incurred

with the production of that phenotype, but these costs are not true costs of plasticity. For example, if a tadpole genotype had high plasticity in size at metamorphosis (regardless of the range of sizes attainable) at the expense of either plasticity in fat storage or the ability to attain fat levels that maximize fitness, then the plasticity could be said to have a cost over and above the pure costs of small body size. Newman (1988a) found a cost of plasticity in spadefoot (Scaphiopus couchii) tadpoles; among families, higher plasticity in growth (not simply larger size at metamorphosis) was associated with slower development.

I measured plasticity of P. crucifer families across the four feeding treatments in units of standard deviations of the family grand mean (Table 2-4) (Leips and Travis 1994). Families with higher plasticity in growth show slower, less plastic rates of development, just as they did in Newman's (1988a) study. In addition I found that greater plasticity in lipid storage was associated with slower, less plastic development and larger, more plastic size at metamorphosis. Newman's study was limited to just 5 sibships (Newman 1988a), and my data represent just four, making all conclusions tentative. If such a trade-off between plasticity in growth and plasticity in development rate is real, and not an artifact of small sample sizes, Newman (1992) suggested that this trade-off is probably the result of the functional relationship between growth and development. Previous workers have suggested a cause-effect relationship between

growth and development in tadpoles, but have differed on which of the two is causal of the other (Wilbur and Collins 1973, Smith-Gill and Berven 1979, Stearns and Koella 1986). The details of the relationship between changes in growth rates and changes in development rates have only recently been explored (Hensley 1993, Leips and Travis 1994). Further research is necessary to determine if there is a physiological basis for a trade-off between plasticity in growth and plasticity in development.

Compensation

Leips and Travis (1994) proposed a model of dynamic allocation of energy in tadpoles. According to this model, during early developmental stages energy is primarily allocated toward development, and growth is a lesser priority. Changes in energy income in this phase of larval ontogeny primarily affect timing of metamorphosis, but also affect growth. Beyond some boundary, however, allocation patterns change, and fluctuations in energy cease to affect development rates but strongly affect size at metamorphosis.

In the present study food level manipulations were expected to affect both size at metamorphosis and timing of metamorphosis, but no effect on timing was realized. Feeding groups L and D did not suffer delayed metamorphosis, but metamorphosed with lower levels of stored fat than did animals that had high food availability (I, H) in later

development. This result may suggest that tadpoles raised on low food allocated energy preferentially to maintain rapid development and never fell behind. An alternative is that family effects and block (temperature) effects simply swamped any treatment effects on development rate, and thus obscured any underlying relationship between energy allocation and rapid development. Further studies of the details of growth, development, and energy allocation are necessary to determine whether the lack of a developmental response was actually due to dynamic allocation or was simply the result of high within-family variance in development rates.

Growth of the food increase group (I), however, suggests that tadpoles are able to compensate for low food in early development, without significant delays in metamorphosis or reduction in lipid storage. One possible explanation is that low food levels in early larval stages can condition tadpoles to be more efficient at energy accumulation, and thus makes them better able to take advantage of the increase in food resources and "catch up" to the high food group. Alternatively, these tadpoles may be paying some cost of reduced early growth that is simply undetected. Unfortunately, both of these arguments assume that the first two weeks of growth resulted in differences between the high and low food groups, but in this experiment I did not measure tadpoles prior to the food switch. Possibly food was not a limiting factor during the first two weeks of development, even on the low food ration. Data from a previous study on

this species (Hensley 1993), however, suggest that significant differences in body size between the low and high food rations should have been attained by the fourteenth day of the experiment. The ability of tadpoles to change energy allocation to compensate for reduced growth and development when resource levels increase warrants further study.

The results of this study indicate that energy allocation is a phenotypically plastic trait in tadpoles. Full sibling families differed in the degree of plasticity in growth, development, and lipid storage. My data support Newman's suggestion that plasticity in development may be traded against plasticity in growth (and energy storage). As proposed by Crump (1981), energy accumulation is an important component of the larval period for tadpoles. Models for predicting metamorphosis may be improved by considering energy accumulation and allocation, but more detailed research on the relationship between growth and development is necessary for a clear picture of the role of energy accumulation and allocation. The ability of tadpoles to compensate for early limitations on growth and development and the potential costs of compensatory growth and development also warrant further exploration. Genetic trade-offs between rapid growth and developmental plasticity are also suggested as elements that must be considered for a full understanding of the adaptive significance of plasticity in tadpoles.

Table 2-1. Characteristics of female P. crucifer and their egg clutches.

sibship	♀ SVL (mm)	egg dia (mm) $\bar{x}$ ± s.d.	$\bar{x}$ egg dry mass (mg)	eggs laid	total clutch	% lipid in eggs
1	32.5	1.20 ± 0.023	0.51	350	926	84.6
2	28.5	1.04 ± 0.020	0.34	446	800	88.0
3	32.0	1.14 ± 0.022	0.45	277	1056	93.3
4	33.8	1.14 ± 0.021	0.43	253	1270	81.6

Table 2-2. Results of MANOVA and univariate ANOVAs for age at metamorphosis, dry mass at metamorphosis, and lipid reserves for Pseudacris crucifer tadpoles. Wilks' λ is the multivariate test statistic. Probability values < 0.05 are indicated with an asterisk (*). Coefficients of determination (r^2) indicate the proportion of total variance explained by each factor.

MANOVA				
Source	Wilks' λ	F	df	P
treatment	.5379	9.891	9, 306.80	0.0001*
sibship	.6960	5.475	9, 306.80	0.0001*
shelf	.3465	7.705	21, 362.35	0.0001*
treatment x sibship	.8318	0.888	27, 368.63	0.6292
sibship x shelf	.6799	0.832	63, 376.95	0.8122
treatment x shelf	.5609	1.279	63, 376.95	0.0869

	larval period	dry mass	% lipid
Source	P	P	P
treatment	.5584	.0001*	.0001*
sibship	.0001*	.1958	.0086*
shelf	.0001*	.3911	.9778
treatment x sibship	.5838	.9343	.2017
sibship x shelf	.2015	.9556	.7102
treatment x shelf	.4152	.0096*	.1000
coefficient of determination (r^2)	.7144	.5826	.5347

Table 2-3. Analysis of covariance for percent lipid in Pseudacris crucifer tadpoles. Type I sums of squares test sequential hypotheses explained in text.

Source	df	Type I SS	MS	F	P
sibship	3	.0121	.0040	8.12	.0001*
dry mass	1	.0696	.0696	139.67	.0001*
(dry mass)^2	1	.0010	.0010	1.94	.1653
treatment	3	.0066	.0022	4.43	.0049*
residual	188	.0936	.0005		

Table 2-4. Mean responses and phenotypic plasticity in larval period, size at metamorphosis, and lipid storage in P. crucifer tadpoles. Plasticity is the maximum difference in treatment means (in units of standard deviation of the family mean).

	larval period (days)		dry mass (g)		lipid (% dry mass)	
sibship	$\bar{x}$	plast.	$\bar{x}$	plast.	$\bar{x}$	plast.
1	41.60	0.3200	.0324	1.4928	9.67	1.5559
2	39.27	0.6857	.0305	1.3654	10.55	1.2270
3	39.00	0.8716	.0314	1.3182	10.74	0.9842
4	39.75	0.5864	.0328	1.3538	10.49	1.0340

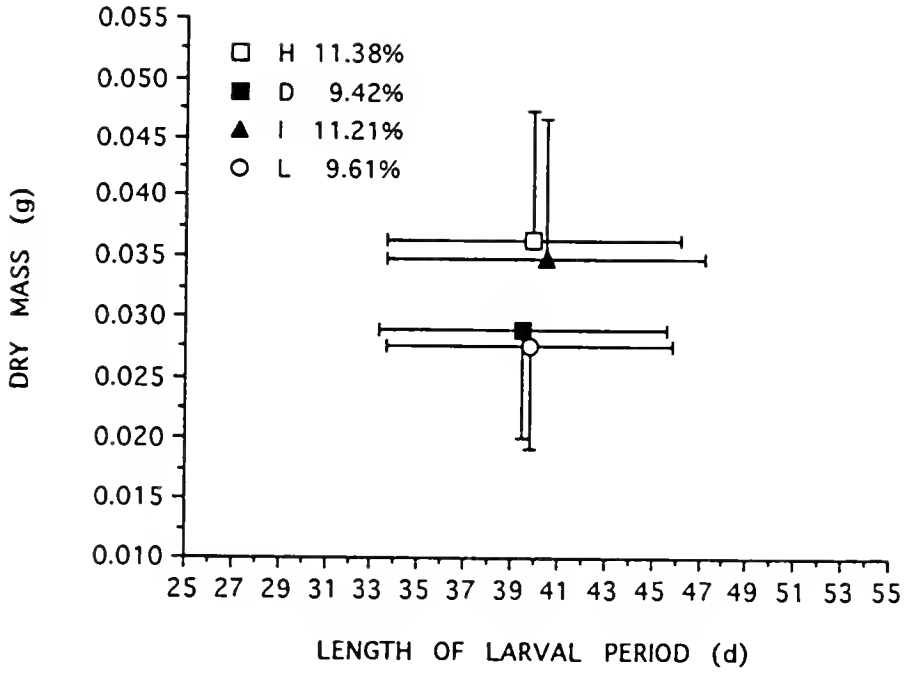

Figure 2-1. Treatment means for age and dry mass at metamorphosis for P. crucifer tadpoles. Mean lipid percentages are shown for each treatment. Control groups were raised on constant high (H) or low (L) food levels. Food increases (I) and decreases (D) were made after 14 days on initial food levels. For clarity only one side of the 95% confidence intervals for size at metamorphosis are shown.

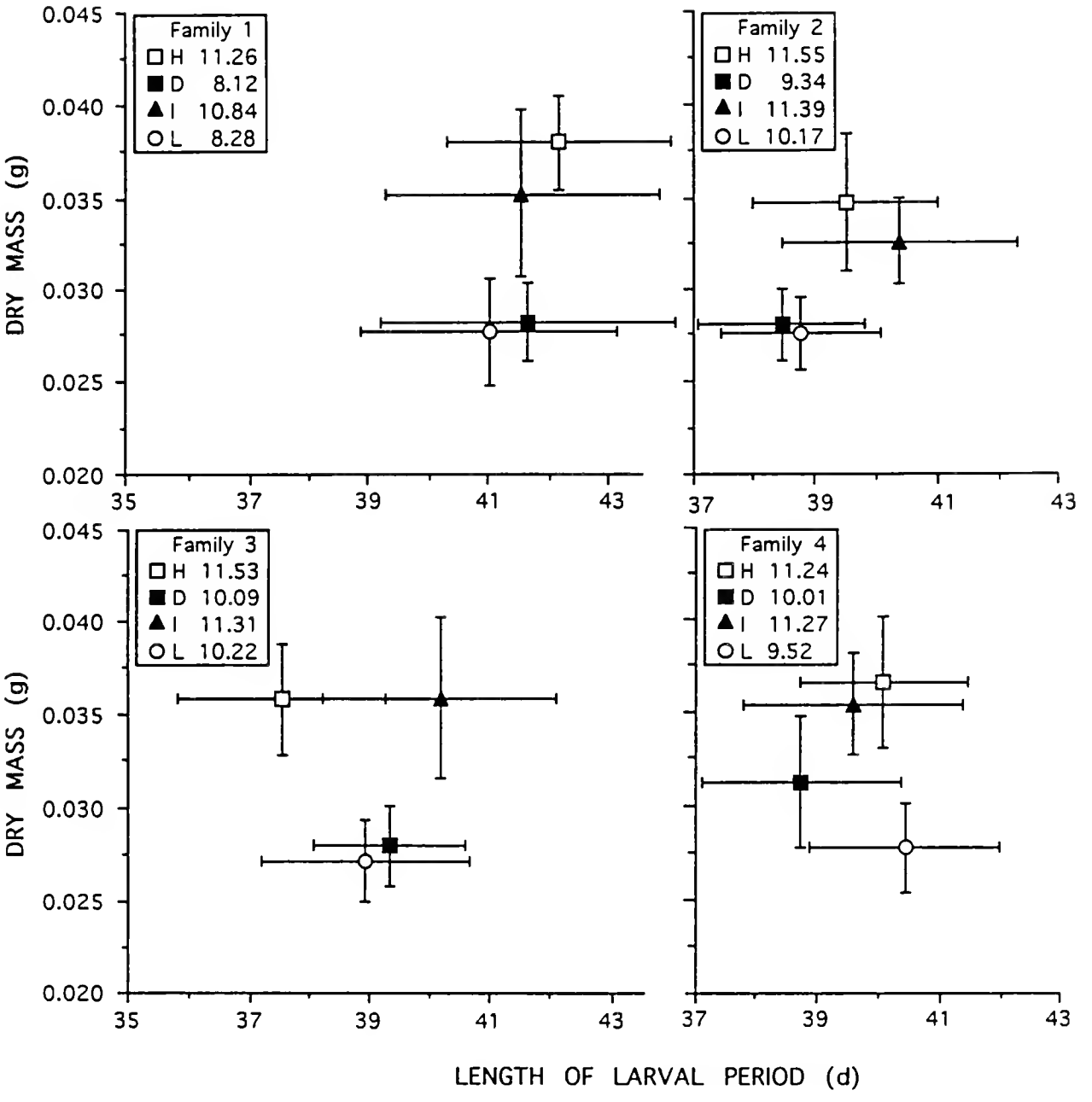

Figure 2-2. Mean responses to the four feeding treatments for each sibship with mean lipid percentage given in each legend. All plots are made to the same scale.

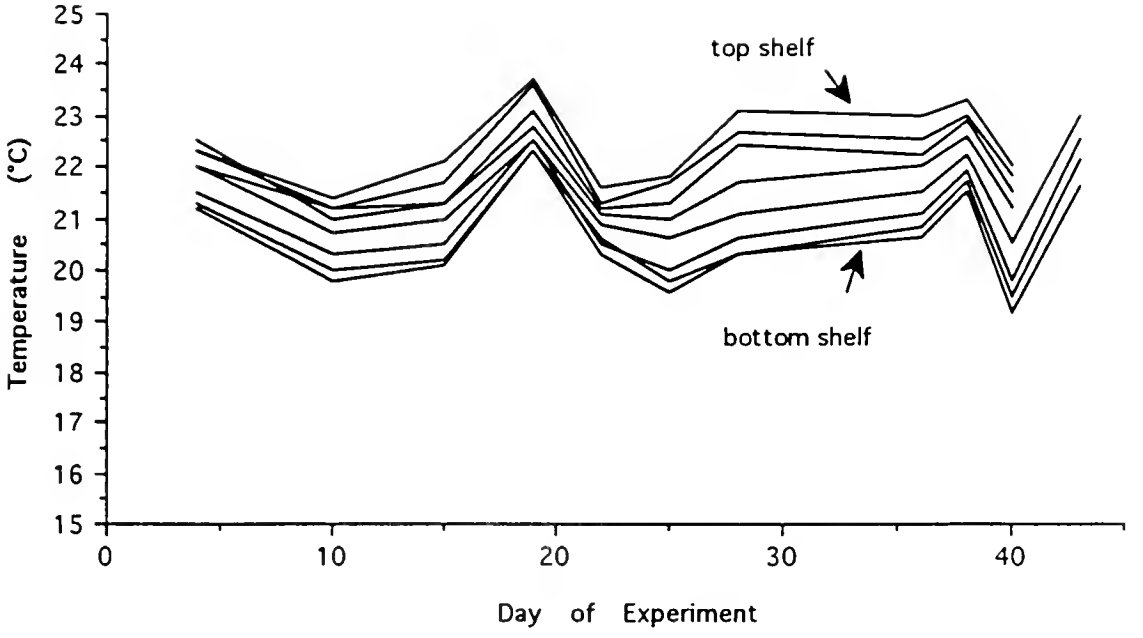

Figure 2-3. Water temperature measurements for each shelf, measured 11 times during the experiment.

CHAPTER 3
GROWTH AND DEVELOPMENTAL TRAJECTORIES OF INDIVIDUAL TADPOLES IN RESPONSE TO CHANGING FOOD SUPPLY

Introduction

Phenotypic plasticity of growth and development in amphibian larvae has received much attention in experimental ecology over the last two decades. This research has focused on two central questions. First, given the wide ranges of sizes and ages at which larvae metamorphose within populations, can we predict when metamorphosis will occur for individual larvae (reviews in Alford and Harris 1988, Hensley 1993)? Second, is phenotypic plasticity in amphibian larvae adaptive; that is, does plasticity in the timing of metamorphosis result in greater fitness across a range of environments than could be attained by fixed timing (reviewed by Newman 1992)? Central to answering both of these questions is an understanding of the functional relationship between growth and development. Previous work has treated growth as a prerequisite of developmental change (Wilbur and Collins 1973, Stearns and Koella 1986), but the converse has also been argued (Smith-Gill and Berven 1979).

Wilbur and Collins (1973) developed a model to predict how growth and development rates of amphibian larvae are related, and how changes in growth rate influence age and size at metamorphosis. This model has become central to our understanding of phenotypic plasticity in complex life cycles (life cycles that include a larval stage followed by a radical metamorphosis and usually a habitat change (Wilbur 1980)).

The Wilbur-Collins model proposes that amphibian larvae must reach a minimum size threshold (b) before successful metamorphosis is possible. Above that size, metamorphosis is initiated when the mass-specific growth rate falls below a threshold level (g). Based on these two principles, we can predict how development rates will be affected when changing conditions in the larval environment induce changes in growth rate. In the early stages of development when tadpoles have not yet reached the size threshold (b), increased growth rate will allow tadpoles to reach b earlier and thus result in earlier metamorphosis, but decreased growth rate will delay metamorphosis. Once tadpoles have crossed the size threshold, however, the opposite developmental response is predicted: increased growth rate will delay the time when mass-specific growth falls below g, and thus will delay metamorphosis. Above size b, a decrease in growth rate should accelerate metamorphosis.

Experimental tests have generally supported the Wilbur-Collins model (Alford and Harris 1988, Hensley 1993, Leips

and Travis 1994, Tejedo and Reques 1994), but raise questions about the details of the correlations between growth and development. For example, Hensley (1993) found that development rates become fixed in the latter third of the larval period and do not respond to subsequent changes in growth rate. Leips and Travis's (1994) study confirms this observation, and they proposed that during larval development tadpoles allocate energy to either growth or development, but the pattern of allocation changes over time.

According to their model (Leips and Travis 1994), growth and development are competing functions, and energy allocated to one function represents a trade-off with allocation to the other. During development, however, a tadpole's allocation priorities change. In the early stages of larval life allocation of energy to development takes precedence over allocation to growth, but the importance of allocation to development declines over time. Eventually, at the point when developmental timing becomes fixed (Hensley 1993), all excess energy is allocated to growth. Leips and Travis's (1994) model predicts asymmetric responses to changes in food supply. Early in development a decrease in food supply is predicted to affect growth first, because development rate is maintained as a high allocation priority. For tadpoles on low food, an early increase in food will first accelerate development rate, and only after development rate is maximized will growth be accelerated.

At later larval stages, however, allocation to growth becomes the higher priority, developmental responses to changing food supply are reduced, and eventually development rate ceases to respond to changes in food supply (Hensley 1993). According to Leips and Travis's (1994) model, later increases in food result in larger size at metamorphosis because development places less demands on energy later in the larval period. This prediction that later food increases result in greater size increases is contrary to the Wilbur-Collins model.

In this model of dynamic allocation (Leips and Travis 1994), growth and development are competing (i.e., negatively correlated) functions; the strong positive correlation generally seen between growth and development may be due to both functions being highly correlated with age (Bernardo 1993). Comparisons among this model, the Wilbur-Collins model, and experimental studies (Alford and Harris 1988, Hensley 1993, Tejedo and Reques 1994), raise questions about the details of this ontogenetic change in priorities.

At what stage does this change in priorities occur? Hensley (1993) proposed that by stage 35-37 (Gosner 1960) tadpole development rates do not respond to changes in growth rate. Whether this point in development marks an abrupt shift in energy allocation priorities or the end of a gradual transition is unclear. Further, how much plasticity in development is seen in the early and middle stages of larval ontogeny? Are development rates particularly sensitive at

certain developmental stages, and less sensitive at other stages?

Answers to these questions require data on the ontogeny of individual larvae raised under a variety of constant and changing conditions. Yet, to date, only a single study has monitored the development of individual tadpoles in an experimental setting (Smith-Gill and Berven 1979). That study, however, did not examine the effects of changing food supply on the timing of metamorphosis. Other experimental studies have focused on growth trajectories and timing of metamorphosis, ignoring the details of developmental trajectories.

I performed an experiment to examine the details of developmental response in individual Bufo terrestris tadpoles reared in variable growth environments. The goal of this experiment was to characterize the relationship between growth and development, and to ascertain whether the dynamics of this relationship effectively predict individual differences in the timing of metamorphosis.

Methods

This experiment was designed to generate variation in growth trajectories of tadpoles and to make comparisons among tadpoles in the developmental responses to changes in growth rate. Tadpoles were raised individually on controlled food rations from hatching to metamorphosis. Some tadpoles were

raised on constant food levels (control treatments), while others experienced either increases or decreases in food availability during the experiment. For each tadpole, I recorded wet mass and developmental stage seven times during the larval period. Because genetic variation in larval growth and development has been demonstrated for many amphibians (Travis 1980, Newman 1988b, Semlitsch et al. 1990) I used two separate full-sibling families in this experiment.

This experiment tested the responses of tadpoles to eight different feeding regimes. I established two feeding levels (high and low) used throughout this experiment. Feeding treatments included two constant food level treatments (controls, H and L) raised on the high and low feeding rates, respectively. In addition six variable food treatments were switched between high and low feeding rates. Treatments I1, I2, and I3 experienced increased food levels, switching from low to high food on one of three different days (12, 18, 24) during the experiment. The reciprocal food decreases were made on the same schedule, designated as treatments D1, D2, and D3.

Bufo terrestris (Bufonidae) breeds in both temporary and permanent ponds from spring to fall, and thus experiences a variety of unpredictable environments in nature. On 21 April 1992 I collected two amplectant pairs of toads from a roadside ditch on the Savannah River Site, Aiken County, South Carolina. Each pair was housed in a plastic container with shallow water and loose vegetation from the collection

site. Both pairs laid eggs, but hatching success was much higher in sibship 1 than in sibship 2. On 28 April I haphazardly selected tadpoles from each clutch and randomly assigned them to treatments.

Tadpoles (N = 256) were raised individually in plastic cups on 8 laboratory shelves, which were treated as spatial blocks. Each treatment-sibship combination was replicated twice per shelf, and cups were randomly assigned positions on shelves. Cups were 9.5 cm tall x 9.2 cm diameter and were filled with 370 ml of well water. Water was changed prior to each feeding. Tadpoles were fed every third day. Food was delivered from a glass jar with a perforated metal lid. Each shake of the jar delivered 13.3 mg (c.v. = 14.6%) of food. The diet consisted of a finely ground mixture (1:1 by mass) of Purina® rabbit chow and NutraFin® fish flakes. Tadpoles were either fed a high food level (2 shakes per feeding) or a low food level (1 shake per feeding).

Throughout this experiment the growth and development of individual tadpoles were monitored. Logistics of weighing and determining developmental stages necessitated dividing the experiment into two temporally staggered halves. Tadpoles on odd numbered shelves were always weighed, staged, and fed one day earlier than tadpoles on even numbered shelves. Tadpoles were first fed on 28 April (designated day 0). Tadpoles on odd numbered shelves were next fed on day 3; tadpoles on even shelves were fed on day 4, and this 1-day difference was maintained throughout the experiment.

Henceforth all references to treatment and data collection schedules are for the odd shelf regime, but even shelves were always treated identically, one day later. Food increases (I1, I2, and I3) were made on days 12, 18, and 24, respectively. Food decreases (D1, D2, D3) were made on this same schedule.

Tadpoles were weighed and developmental stages determined on days 9,12,18,24,30. All growth and developmental data were pooled for each pair of days (e.g. wet masses and stages measured on days 9 (odd shelves) and 10 (even shelves) were treated as simultaneous measurements). To weigh tadpoles I blotted them with a moist paper towel to remove excess surface water and weighed them to the nearest 0.1 mg in a tared beaker of water. Gosner (1960) developmental stages were determined by examining each tadpole in a transparent vial of water under a dissecting microscope. The vial was held horizontally and rotated about its long axis until the tadpole's hind limbs became visible. Vial size was chosen to limit tadpole movement, and thus depended on tadpole size.

For each tadpole, date and mass at forelimb emergence (stage 42) and at tail resorption (stage 46) were recorded.

Statistical Analyses

All statistical analyses for this experiment were performed with SuperANOVA® 1.1 software (Abacus Concepts Inc.

1989). I used two repeated measures analyses of variance (ANOVAs) to test for treatment and sibship effects on growth and developmental trajectories. Mass measurements were $\log_{10}(x+1)$ transformed to meet the assumption of homogeneous variances. Growth trajectories included measurements of wet mass on days 9, 12, 18, 24, 30 and wet mass at Gosner stages 42 (fore limb emergence) and 46 (complete tail resorption). Using mass at stages 42 and 46 as the final two growth observations standardizes the trajectories for differences in development rate.

Developmental trajectories included Gosner stage observations on days 9, 12, 18, 24, 30, plus the number of days to reach Gosner stages 42 and 46. Using time to Gosner stages 42 and 46 as the endpoints of these trajectories, rather than a uniform date of observation, means that the entire larval period of each tadpole was included in the analysis, regardless of length of the larval period.

These ANOVAs were followed by *a priori* planned contrasts (df = 1) between each control (H, L) and each manipulated treatment (D1-3, I1-3) to test for differences in mass averaged over time and developmental stages averaged over time. The interaction of each of these contrasts (control vs. experimental) with time was also calculated to test whether the differences between control and treatments were consistent over time. These interaction tests provide a quantitative assessment of variation in trajectory shape.

Repeated measures ANOVAs provide powerful, formal tests for overall effects of feeding treatment and sibship effects on growth and development, but do not test for differences in mass or developmental stage on each day of observation. To test for such differences, I analyzed the same growth and developmental trajectories using two multivariate analyses of variance (MANOVAs) followed by Dunnett's tests to compare both controls to manipulated treatments on each day of observation. For each day's comparison the experimentwise error rate is $\alpha = 0.05$, but due to the number of tests being made (7 for growth, 7 for development) interpretation is limited to description of trends over the course of the experiment.

Results

In this experiment 200 of the original 256 tadpoles metamorphosed. Survivorship in each treatment ranged from 19 to 29 of the original 32 tadpoles, with the lowest survivorship in both families occurring in the D1 treatment. Sibship 1 had 105 (82%) survivors and sibship 2 had 95 (74%).

Growth Trajectories

Figures 3-1 and 3-2 show growth trajectories and mean metamorphic responses for the eight feeding treatments. The repeated measures ANOVA (Table 3-1) revealed highly significant effects of feeding treatments, sibships, and

blocks (shelves) on growth trajectories. Sibships differed significantly in overall growth, but not in growth trajectory shape, as indicated by a lack of significant time x sibship interactions. Block x time and treatment x time interactions indicate that growth trajectory shapes differed across treatments, as intended, and across blocks.

Pairwise contrasts of growth between all control-treatment pairs indicate that food switches significantly affected growth (Table 3-2). Contrast interactions with time indicate that except for L vs. D1 and H vs. I1, all experimental growth trajectories had significantly different shapes from both controls (Table 3-2).

Dunnett's tests for significant differences on each day of observation suggest that prior to the food increases, treatments I1-I3 were not different in mass from the control L (Table 3-3). In each case, however, by the next weighing (six days after the food increase), the manipulated treatments were significantly larger than the low food control. In early growth these tadpoles were significantly smaller than the high food control, but by Gosner stage 42 this difference in size had been made up by the first and second food increase treatments (I1-2). Throughout development the third increase treatment (I3) remained significantly smaller than the high food control.

Prior to the food manipulations, none of the decreased treatments (D1-D3) differed from the high food control (H), according to Dunnett's tests. By six days after the food

decrease, each treatment was significantly smaller than H, and remained so through metamorphosis. By day 12 of the experiment, treatments D1-3 were significantly larger than the low food control, L. After the food decreases, however, all three treatments grew slowly and the low food control caught up, resulting in no significant differences in size at metamorphosis (stage 42).

Developmental Trajectories

Figures 3-3 and 3-4 show developmental trajectories for the eight feeding treatments. Repeated measures ANOVA revealed significant effects of treatments, sibships, and blocks on developmental trajectories (Table 3-4). Interactions with time revealed that trajectory shapes were significantly influenced by both sibship and feeding treatment, but not by the block (shelf) effect.

Contrasts revealed that the H and L controls did not develop at significantly different rates, nor did their developmental trajectories differ in shape (Table 3-5). In general, development rates of manipulated treatments were very similar to controls (Table 3-5), with only the earliest decrease (D1) significantly affecting developmental timing compared to the controls. Time interaction contrasts (Table 3-5) showed that the developmental trajectory of D1 took a different shape from that of the high food control. A difference in trajectory shapes was seen in the comparisons

of D3, I1, and I2 to L, but these treatments did not differ in overall developmental timing.

Results of the Dunnett's tests for day by day differences in development suggest that prior to food manipulations the food increase treatments did not differ from the low food control (Table 3-6). The first food increase treatment, I1, accelerated development in response to the change in food supply, but did not maintain this advantage, and the L treatment caught up. This result is consistent with the significant contrast for trajectory shape between these L and I1 (Table 3-5). Dunnett's test indicated that treatments I2 and I3 initially fell behind the H control (Table 3-6), then caught up by metamorphosis. Overall these differences were not significant according to pairwise contrasts (Table 3-5).

For the food decrease treatments, Dunnett's test is not as helpful in interpretation. Although L and D1 differed significantly in overall development (Table 3-5), Dunnett's test does not indicate any significant day-to-day differences (Table 3-6). Significant differences between D2 and L indicated by Dunnett's tests are not reflected in the contrasts (Table 3-5). They suggest, however, that D2 was developing more rapidly than L prior to the decrease in food, but lost this advantage within 12 days. The developmental advantage was present for at least 12 days, but was slight with respect to overall developmental timing and trajectory shape. Significant differences between L and D3 on days 18-

30 (Table 3-6) contributed to differences in trajectory shape (Table 3-5). Dunnett's test indicated only a single significant difference between the H control and a food decrease; D1 was different on day 18. The contrasts, however, indicated that these two treatments developed at marginally different rates (P = .0515) and had extremely different trajectory shapes.

Discussion

This experiment explores the details of the relationship between developmental trajectories and growth trajectories and tests the model of dynamic allocation (Leips and Travis 1994). According to the dynamic allocation model, early manipulations of food supply should affect development rates more strongly than later manipulations. In Bufo terrestris experimental manipulations of food supply revealed that earlier changes in food supply had larger effects on developmental timing. Later food increases were predicted to result in greater size increases (Leips and Travis 1994), but this prediction was not supported. Results with B. terrestris thus provide some limited support for the hypothesis of changing allocation priorities.

A comparison between the H and L controls illuminates the changes in allocation priorities. Although body sizes of H and L diverged by day 9 of the experiment and remained different through metamorphosis (Table 3-3), developmental

trajectories were not significantly different overall (Table 3-5). During days 18-30, however, there is evidence that the H control tadpoles were at significantly more advanced stages of development than L tadpoles (Table 3-6), but this difference did not persist through metamorphosis. These subtle differences in trajectories can be interpreted in terms of changing developmental priorities.

Prior to day 18, food did not limit development, and the two controls (L and H) followed similar developmental trajectories, even though there was enough excess food to allow H to grow much faster than L. This similarity in developmental stages suggests that both groups were maximizing development rate. From day 18-30 H tadpoles used their additional resources to develop significantly faster than L, indicating that development was a high priority during this interval, and that food became a limiting factor for development of L tadpoles. During this time period, L tadpoles grew, indicating that the reduced development compared to H was balanced against allocation to growth. Between day 30 and metamorphosis the developmental difference between the controls disappeared. Either development became less of a priority for H, or the L control underwent a compensatory acceleration of development, or both.

A compensatory acceleration of development by the L control is not consistent with the hypothesis that development becomes a progressively lower priority in later stages. Apparently H tadpoles did not maximize development,

but delayed metamorphosis (compared to the possible trajectories of such groups as I1, I2 and D3) and took advantage of the opportunity for greater growth. This sacrifice of rapid development in favor of greater growth supports the model of dynamic allocation and its hypothesis that allocation to development declines in later stages in favor of allocation to growth.

A second line of evidence supporting dynamic allocation is seen in the developmental responses of the treatments that experienced decreased food supplies. The Wilbur-Collins (1973) model predicts that declining food availability early in development, (i.e., before tadpoles have reached the minimum body size (b)), will delay metamorphosis. Food decreases that occur after tadpoles are larger than b will stimulate accelerated development and earlier metamorphosis. Such a switch is consistent with the hypothesis that development is always the first allocation priority, but is constrained by a threshold body size (Wilbur and Collins 1973) or a minimum energy reserve (Crump 1981). In contrast, the model of dynamic allocation suggests that beyond a certain point in development excess energy is allocated preferentially to growth, and declines in food supply will not affect developmental timing.

In this experiment, D1 metamorphosed significantly later than the H control, as predicted by both models. Treatments D2 and D3 did not differ significantly from the H control, as predicted by dynamic allocation. The deviations of D2 and D3

from H are in opposite directions, as predicted by the Wilbur-Collins model. This pattern suggests that tadpoles reached the minimum size/energy threshold somewhere between stage 34 (approximately when D2 was switched, Figure 3-3) and stage 37 (approximately when D3 was switched). The lack of a significant treatment effect in the latter stages coupled with the predicted trend in treatment responses supports the idea that the dynamic allocation model is complementary to the Wilbur-Collins model (Leips and Travis 1994).

The importance of early growth history to later developmental responses is evident in comparisons of the control treatments and in the responses to food increases. The loss of significant differences in developmental stage between L and H (Dunnett's tests, Table 3-6) may indicate one of three possibilities. First, the L control may have increased allocation to development after day 30 (≈ stage 34) and caught up with H. Second, L and H may both have had fixed development rates (Hensley 1993) beyond day 30, but the low food level may have set the development rate of L higher, resulting in convergence. Third, H may have delayed metamorphosis in favor of greater growth, and thus allowed L to catch up. Of these three alternatives, the first is least likely based on the dynamic model of allocation, since allocation to development is predicted to decline over developmental time. All three explanations, however, may be accommodated by the model, and all suggest that early growth

rates may influence how allocation priorities change over time.

The importance of early growth to later responses is also evident in the effects of food increases. Leips and Travis (1994) stated that the progressive subordination of development to growth as an allocation priority would result in later food increases causing larger increases in size at metamorphosis. Previous studies with temporary-pond breeding species have found the opposite trend; later food increases resulted in smaller size increases (Alford and Harris 1988, Hensley 1990, Hensley 1993, this study). These two patterns may reflect adaptation to temporary versus permanent ponds. For temporary-pond breeders it is possible that the cumulative effects of low food supply result in greater allocation to development in the later stages of the larval period. Late food increases may serve to promote development to a greater degree in species where the risk of desiccation is high, and thus result in smaller size at metamorphosis than early increases. Leips and Travis (1994) interpreted the ability to take advantage of growth opportunities that arise late in the larval period as adaptive for permanent ponds, but maladaptive for temporary ponds.

Leips and Travis (1994) studied Hyla cinerea, a permanent-pond breeder, and H. gratiosa, a closely-related temporary pond breeder. They detected the trend for progressively later food increases to generate progressively larger size at metamorphosis in both species, but the trend

was present only at 25°C, not at 31°C. They attributed this trend to adaptation for permanent ponds, speculating that H. gratiosa and H. cinerea had a common ancestor that bred in permanent ponds. An alternative explanation is that this trend is general for tadpoles growing at lower temperatures, and it was not seen in other studies simply because higher temperatures affected how allocation patterns changed during development. Leips and Travis's (1994) study is the first to examine the interaction of temperature and changes in food supply. They found significant interactions between temperature and feeding treatments on development rates of H. cinerea, but not in H. gratiosa. They found no significant interactions of temperature and feeding treatment on size at metamorphosis. Further factorial experiments that incorporate changes in food supply at various temperatures will be necessary to determine the extent to which this aspect of breeding habitat influences how allocation dynamics change over time.

Hensley (1993) concluded that development rates of Pseudacris crucifer tadpoles became fixed at approximately Gosner stage 35-37, based on a lack of significant differences in timing of metamorphosis between late experimental food switches and controls. The same approach would suggest that for Bufo terrestris the fixation of development occurred by stage 34, since later manipulations did not result in significantly different timing of metamorphosis (Figures 3-3 and 3-4). Analysis of maturation

phenotypes without regard to trajectories that lead to them may obscure dynamic changes during ontogeny (Bernardo 1993). Changes in growth trajectory shape after stage 34 (L vs. H) and direction of response (D2 vs. D3) clearly show that plasticity persists later than would be indicated by analysis of trajectory endpoints alone.

According to the dynamic allocation model (Leips and Travis 1994), early food manipulations should result in greater changes in development rate than later manipulations. An examination of Figure 3-4, however, suggests that the changes in development rates (trajectory slopes) were very similar in magnitude across the entire experiment. I suggest that changes in food availability may have similar effects on development rate at any time during larval development, but that earlier changes simply have more time for cumulative effects to be manifested. If this is the case, then the patterns of age and size at metamorphosis (Hensley 1993, Leips and Travis 1994, this study) may be largely due to fixed patterns of developmental timing rather than to changes in energy allocation priorities.

An additional concern in application of the dynamic allocation model is its assumption that growth and development are functions that compete for allocation of assimilated energy. While metabolic rates are definitely influenced by body size and temperature, there is no direct evidence that more rapid development is more metabolically demanding than slower development. Feder (1982) measured

oxygen consumption of tadpoles and found that developmental stage accounted for only 2% of the variation in metabolic rate in Bufo woodhousei, and never more than 7% in other species. Because growth and development rates of tadpoles tend to be correlated (Wilbur and Collins 1973, Alford and Harris 1988, Hensley 1993), rapid development may be associated with larger body size, and thus greater metabolic efficiency. Energy allocated to growth, therefore, may actually result in reduced developmental costs. The actual energy costs of rapid versus slower development, adjusted for body size, must be measured in order to test this assumption of the dynamic allocation model.

The results of the present study confirm that even when developmental timing is not significantly influenced by environmental conditions, there is a dynamic relationship between growth and development. Development is significantly influenced by changes in food supply early in the larval period (prior to stage 34 in this study). Later changes in growth rate may not significantly affect timing of metamorphosis, but the interaction of growth and development continues to change until at least stage 37. These results suggest that the model of dynamic allocation and the Wilbur-Collins model are complementary and predict how development rates change in response to changing growth rates. Further research is required to ascertain whether specific relationships between growth and development are adaptive in

permanent, predictable environments versus temporary, unpredictable environments.

Table 3-1. Repeated Measures ANOVA for growth trajectories. Growth measurements are explained in the text.

Source	df	MS	F	P	Error term
1 feeding treatment	7	.0124	29.44	.0001	4
2 sibship	1	.0253	59.97	.0001	4
3 block	7	.0019	4.59	.0001	4
4 tadpole(treatment)	183	.0004			
5 time	6	.0842	1147.40	.0001	9
6 time x treatment	42	.0024	32.50	.0001	9
7 time x sibship	6	.0001	0.56	.7671	9
8 time x block	42	.0003	4.34	.0001	9
9 time x tadpole(treatment)	1098	.0001			

Table 3-2. Probabilities for contrasts in total growth and growth trajectory shape. The left side of the table shows probabilities for the hypothesis of no difference in total growth between treatments. The right side shows probabilities for the hypothesis of no interaction of growth with time for each contrast, a test of differences in trajectory shape. The top row shows the contrast between the two controls; lower rows show contrasts between experimental treatments and the controls. Probabilities deemed significant (< 0.05) are indicated by *.

	total growth		trajectory shape	
L vs. H	.0001		.0001	
	vs. L	vs. H	vs. L	vs. H
treatment				
I1	.0001*	.0190*	.0001*	.4290
I2	.0001*	.0001*	.0001*	.0001*
I3	.0001*	.0001*	.0001*	.0001*
D1	.0070*	.0001*	.6923	.0001*
D2	.0001*	.0001*	.0001*	.0001*
D3	.0001*	.0001*	.0001*	.0001*

Table 3-3. Results of Dunnett's tests for body size differences between the two controls, and between experimental treatments and controls. Observations to the right of the bold line were made after the food level changes. Comparisons marked with * are statistically significant ($\alpha = 0.05$). Observation days shown are for odd numbered shelves, but data are pooled for pairs of observation days (see text).

	DAY 9	DAY 12	DAY 18	DAY 24	DAY 30	STAGE 42	STAGE 46
H vs L	*	*	*	*	*	*	*
L vs I1			*	*	*	*	*
L vs I2				*	*	*	*
L vs I3					*	*	*
H vs I1	*	*					
H vs I2		*	*	*	*		
H vs I3	*	*	*	*	*	*	*
L vs D1		*					
L vs D2	*	*	*	*	*		
L vs D3		*	*	*	*		
H vs D1			*	*	*	*	*
H vs D2				*	*	*	*
H vs D3					*	*	*

Table 3-4. Repeated Measures ANOVA for developmental trajectories. Observations of development are explained in the text.

Source	df	MS	F	P	Error term
1 feeding treatment	7	179.77	3.74	.0008	4
2 sibship	1	416.39	8.71	.0036	4
3 block	7	108.45	2.26	.0308	4
4 tadpole(treatment)	183	47.79			
5 time	6	10644.88	348.04	.0001	9
6 time x treatment	42	100.94	3.30	.0001	9
7 time x sibship	6	253.83	8.30	.0001	9
8 time x block	42	29.98	0.98	.5083	9
9 time x tadpole(treatment)	1098	30.58			

Table 3-5. Probabilities for contrasts of developmental timing and developmental trajectory shape. The left side of the table shows probabilities for the hypothesis of no difference in developmental timing between treatments. The right side shows probabilities for the hypothesis of no interaction of development with time for each contrast, a test of differences in trajectory shape. The top row shows the contrast between the two controls; lower rows show contrasts between experimental treatments and the controls. Probabilities < 0.05 are indicated by *.

	developmental time		trajectory shape	
L vs. H	.8843		.3760	
	vs. L	vs. H	vs. L	vs. H
treatment				
I1	.1517	.1106	.0001*	.1989
I2	.0760	.0520	.0036*	.7087
I3	.4336	.3455	.6736	.9956
D1	.0397*	.0515	.0672	.0001*
D2	.1205	.1568	.9852	.0675
D3	.4103	.3238	.0001*	.2153

Table 3-6. Results of Dunnett's tests for developmental stage differences between the two controls, and between experimental treatments and controls. Observations to the right of the bold line were made after the food level changes. Comparisons marked with * are statistically significant ($\alpha = 0.05$). Observation days shown are for odd numbered shelves, but data are pooled for pairs of observation days (see text).

	DAY 9	DAY 12	DAY 18	DAY 24	DAY 30	STAGE 42	STAGE 46
H vs L			*	*	*		
L vs I1			*	*	*		
L vs I2							
L vs I3							
H vs I1							
H vs I2			*				
H vs I3			*	*			
L vs D1							
L vs D2		*	*	*			
L vs D3			*	*	*		
H vs D1			*				
H vs D2							
H vs D3							

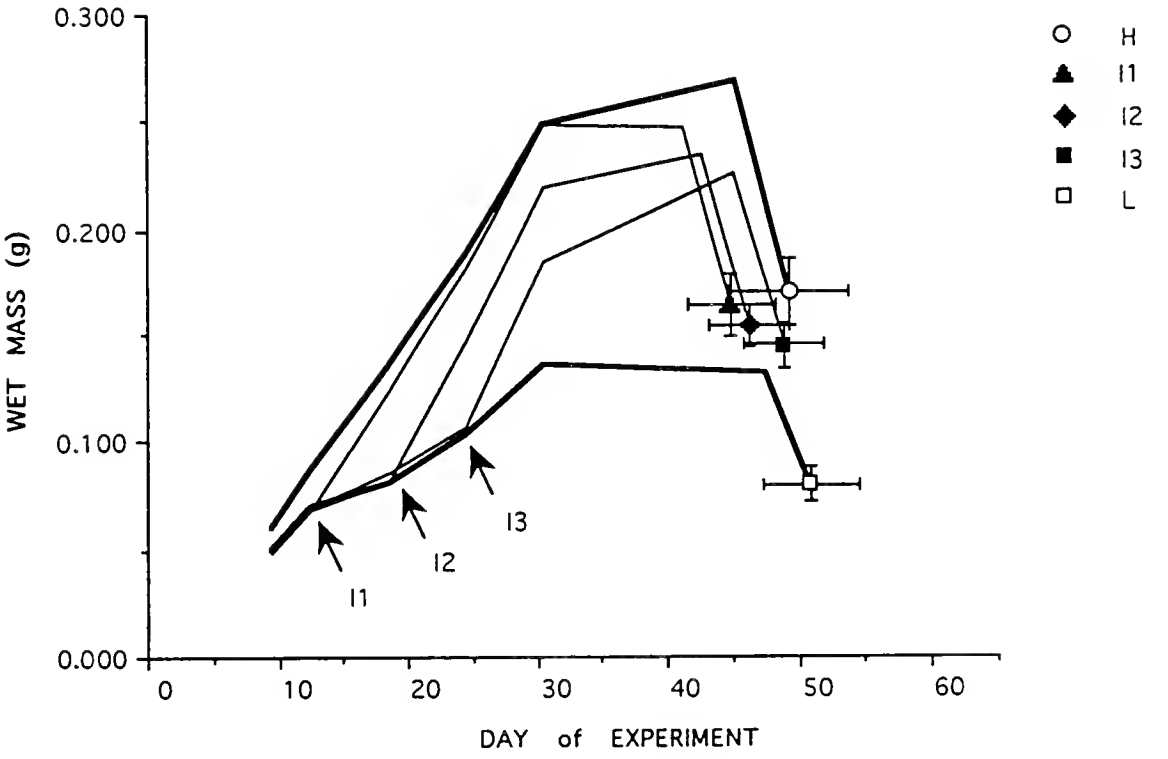

Figure 3-1. Mean growth trajectories for food increase treatments and both controls. Growth trajectories end at mean age and size at metamorphosis (stage 46), shown with 95% confidence intervals. Food level increases are shown by arrows. Weight loss during metamorphic climax is typical for tadpoles and is due mostly to water loss.

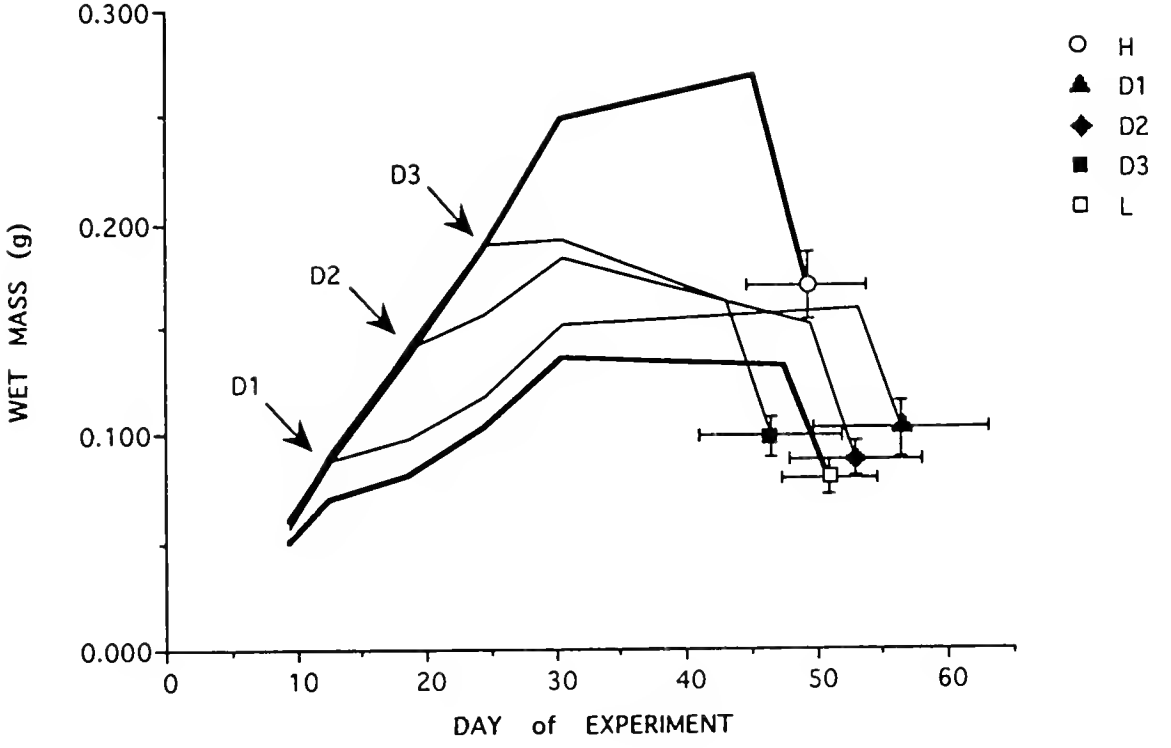

Figure 3-2. Mean growth trajectories for food decrease treatments and both controls. Trajectories end at mean age and size at metamorphosis (stage 46), shown with 95% confidence intervals. Food decreases are indicated by arrows.

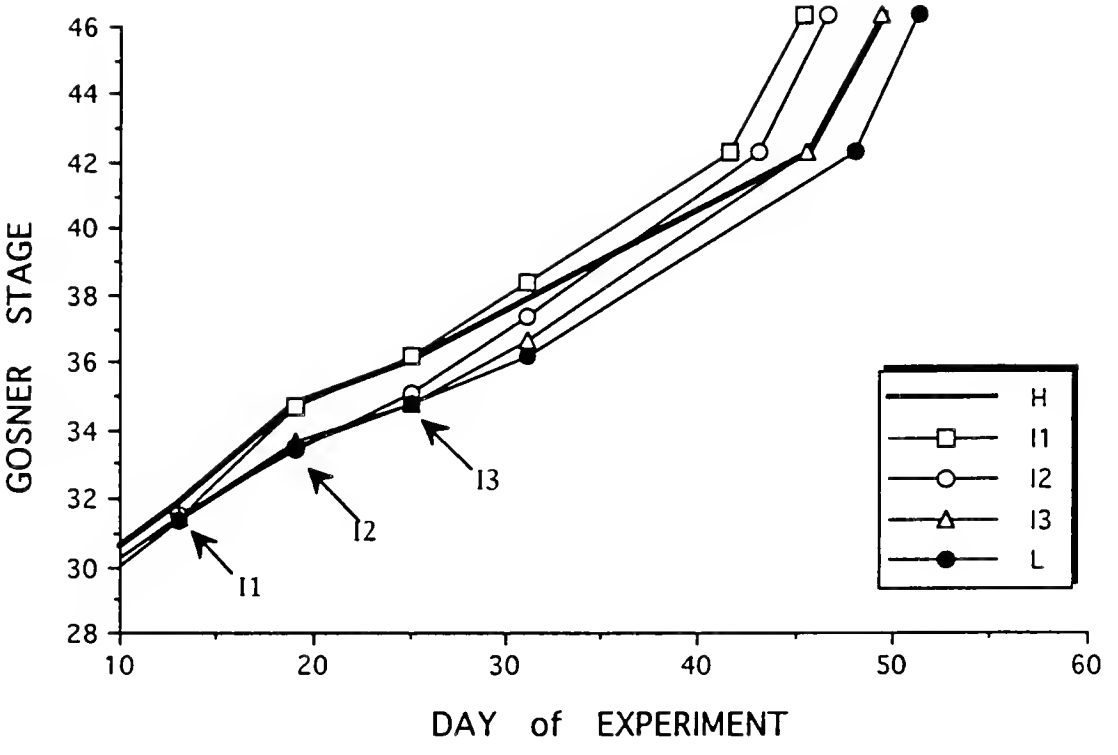

Figure 3-3. Mean developmental trajectories for food increase treatments and both controls. The bold line indicates the high food control (H). The line with filled circles indicates the low food control (L). Food increases are indicated by arrows.

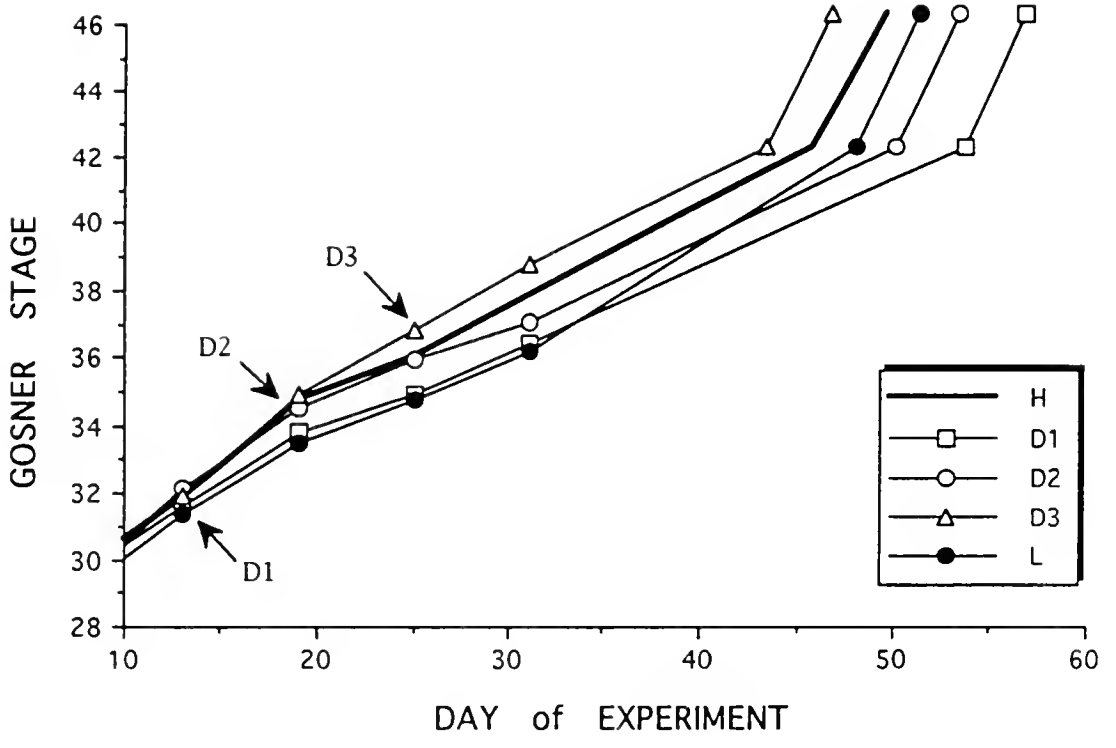

Figure 3-4. Mean developmental trajectories for food decrease treatments and both controls. Control groups are indicated as in Figure 3-3. Food decreases are indicated by arrows.

CHAPTER 4
A MODEL OF STAGE-SPECIFIC ENERGY ALLOCATION DURING GROWTH AND DEVELOPMENT OF AMPHIBIAN LARVAE

Introduction

Age and size at metamorphosis are plastic phenotypic characters in amphibian larvae that have been shown to influence adult fitness (Collins 1979, Smith 1987, Semlitsch et al. 1988, Berven 1990). Wilbur and Collins (1973) proposed a model for predicting age and size at metamorphosis that has become the dominant framework for understanding this plasticity in an ecological context (reviews in Alford 1988, Hensley 1993, Harris *in press*). The Wilbur-Collins model proposes that metamorphosis is possible only for tadpoles above a minimum size, and that metamorphosis is triggered by a reduction of mass-specific growth rate. Thus, environmental conditions that influence growth rate will affect age and size at metamorphosis. Crump (1981) studied energy accumulation in tadpoles of the spring peeper, Pseudacris crucifer, a winter-breeding treefrog. She found that tadpoles raised at low densities accumulated more energy per unit size and developed faster than did tadpoles raised under crowded conditions, even though both groups were fed *ad libitum*. Crump proposed that energy accumulation rate may influence timing of metamorphosis, and that accumulation of a

minimum amount of energy may be necessary for successful metamorphosis.

In a previous study I demonstrated that energy allocation in tadpoles is phenotypically plastic, varying independently of body size (Chapter 2). This plasticity suggests that energy allocation may be a size-independent predictor of metamorphosis. Additionally, growth and developmental trajectories of tadpoles (Chapter 3) are generally consistent with a model of dynamic energy allocation (Leips and Travis 1994) that predicts relative changes in age and size at metamorphosis in response to changes in growth rate. In the present study I test Crump's (1981) prediction that total energy accumulation is an important predictor of the timing of metamorphosis. In addition, I examine the relationships among growth, development rate, and lipid storage in light of a model of proportional allocation of energy, and extend this model to include allocation to storage.

Methods

I performed an experiment to examine the relationship between development rate and lipid storage. The details of the methods are presented in Chapter 3, along with an analysis of resulting growth and developmental trajectories. In this experiment Bufo terrestris tadpoles were raised

individually in plastic cups on controlled food rations from hatching to metamorphosis.

This experiment tested the responses of tadpoles from two sibships to eight different feeding regimes. Feeding treatments consisted of two constant food level treatments (low food (L) and high food (H), considered as controls) and six variable food treatments. Treatments I1, I2, and I3 experienced increased food levels, switching from low to high food on one of three days during the experiment (days 12, 18, and 24, respectively). The reciprocal food decreases were made on the same schedule, designated as treatments D1, D2, and D3.

For each tadpole, age and size at metamorphosis were recorded. Based on the order in which they metamorphosed, every third individual was then assigned to another study (Hensley, *unpublished*). The remaining two thirds of the metamorphs were retained for the present analysis of energy allocation. Lipid storage at metamorphosis was measured using petroleum ether extraction (methods modified from Reznick and Braun (1987), presented in Chapter 2).

Statistical Analysis

Repeated measures analyses of variance on tadpoles in this experiment revealed significant effects of feeding treatments on the shapes of growth and developmental trajectories (Chapter 3). Overall, however, timing of

metamorphosis was very uniform, with only a single treatment (D1) deviating significantly from its control group (H). Body size (mass at metamorphosis), however, was strongly influenced by treatment effects. Based on a previous analysis of lipid storage in tadpoles (Chapter 2), I anticipated that tadpole size would account for most of the variation in lipid storage. To test whether feeding treatments significantly influenced lipid storage, it is necessary to account first for size effects, and also for differences among sibships. I therefore calculated a general linear model using Type I sums of squares that accounted first for body size and sibship effects before testing if treatments significantly affected lipid storage and whether lipid storage was related to development rate. All statistical calculations were performed using SuperANOVA® 1.1 software (Abacus Concepts Inc. 1989).

Results

In this experiment 200 tadpoles metamorphosed, and 142 were selected for lipid extraction. Growth trajectories and mean metamorphic responses for each family are shown in Chapter 3 (Figures 3-1 through 3-4). The relationship between mass and lipid reserves at metamorphosis for each treatment is plotted in Figure 4-1. The general linear model using Type I sums of squares (Table 4-1) showed that size at metamorphosis (dry mass) and sibship explained significant

variation in lipid storage. After accounting for these effects, feeding treatments were marginally significant ($P = 0.085$). The final parameter in the model, day of metamorphosis, was negatively related to adjusted lipid storage (Table 4-1). This relationship is unambiguously negative (slope = -5.39E-6, $P = 0.0002$) but is not strong ($R^2 = 0.087$, Figure 4-2).

Discussion

Crump (1981) proposed that incorporating energy allocation patterns into models of amphibian metamorphosis might increase their power for predicting timing of metamorphosis. In Crump's study, high energy density (J/g body mass) was associated with both large size at metamorphosis and rapid development. This positive correlation between growth and development rates is commonly observed (Wilbur and Collins 1973, Collins 1979, Alford and Harris 1988, Hensley 1993). In the present study, however, early metamorphosis was not associated with large body size (Chapter 3). There is evidence in the present study that early metamorphosis was associated with greater lipid storage, independent of body size (Figure 4-2). Although the relationship was weak, it provides some support for the hypothesis that energy accumulation rate influences development independently of the effects of growth rate.

Leips and Travis (1994) proposed a model of dynamic energy allocation to explain variation in age and size at metamorphosis. According to their model, growth and development are competing functions, and energy allocation between these two functions determines age and size at metamorphosis. In their model, early in the larval period development is the highest priority for allocation. Increases in food supply in this phase of the larval period are primarily allocated to more rapid development, and decreases in food supply result primarily in reduced allocation to growth. Over developmental time, however, growth becomes an increasing allocation priority and the importance of development declines. Late in the larval period changes in food supply affect size at metamorphosis, but developmental timing is fixed and does not respond.

This model of dynamic allocation does not distinguish between absolute energy allocation (Joules) and proportional energy allocation (percent of available energy), nor does it include an energy storage component. Harris (*in press*) has proposed an alternative to Leips and Travis's (1994) model that distinguishes between the absolute amount of energy allocated and the proportional expenditure (Figure 4-3). According to this model both the absolute amount of energy and the proportion of total energy allocated to development increase over developmental time for all tadpoles. Early in development a greater proportion is allocated to growth, but over time this proportion decreases as developmental demands

increase. According to this model, tadpoles in high food environments allocate more energy to development than do tadpoles with less food, but this represents a smaller proportion of their total energy. Thus, tadpoles on high food are able to grow and develop more rapidly than tadpoles on low food.

In Harris's model, changes in food availability will affect age and size at metamorphosis predictably, based on allocation patterns. An increase in food supply that occurs prior to initiation of metamorphosis will make more energy available for both growth and development, resulting in earlier metamorphosis at a larger size. Decreases in food supply prior to initiation of metamorphosis will reduce the total amount of energy allocated to both growth and development, and will decrease the proportion of that energy allocated to growth versus development, resulting in longer larval period and reduced size at metamorphosis.

After initiation of metamorphosis, however, changes in food supply will have different effects (Figure 4-3E). According to Harris (*in press*), at initiation of metamorphosis both the developmental trajectory (timing of metamorphosis), and the energy required to complete development are fixed. This energy demand is dependent on a tadpole's previous growth and is larger for tadpoles from environments with higher food availability. Therefore, when tadpoles experience a food increase after initiation of metamorphosis, a large fraction of the newly available energy

is allocated to growth and results in increased size at metamorphosis. For tadpoles that experience a decline in food late in the larval period, the proportional demands of development increase and result in reduced, and possibly negative, growth.

The foregoing model (Harris *in press*) distinguishes between total energy expenditure and proportional energy expenditure, but also lacks an energy storage component. Harris states that the energy demands of the metamorphic period (from initiation through metamorphic climax) are fixed at the time of initiation and are dependent on a tadpole's recent food availability. Though not explicitly stated, this dependence probably represents the combined effects of body size and developmental stage. This period of fixed allocation and expenditure is based on observations that development rate becomes insensitive to changes in growth rate (Hensley 1993, Leips and Travis 1994). Analysis of developmental trajectories (Chapter 3) indicates that a lack of differences in timing of metamorphosis is not necessarily indicative of a lack of developmental plasticity, and thus may not represent fixed allocation to development.

Crump (1981) first suggested that a minimal energy reserve was probably a prerequisite for successful metamorphosis, and proposed that fat stores were a likely source of such energy. I suggest that ontogenetic changes in allocation to fat storage are complementary to Harris's (*in press*) model and can explain the patterns of lipid storage

seen in Pseudacris crucifer (Chapter 2) and B. terrestris. The results from B. terrestris indicate that changes in food supply at different developmental stages produce differences in allocation to growth versus fat storage. There is a significant trend for allocation to lipids to be greatest if food availability increases at intermediate developmental stages, compared to both earlier and later increases (Figure 4-4). The non-linear response of lipid storage suggests that for tadpoles on low food, lipid storage is an increasing priority relative to body size, up to a point. Later food increases seem to be allocated toward increased body size rather than proportionally larger fat reserves (Figure 4-4). This pattern is consistent with the hypothesis that tadpoles must store some minimum amount of fat for successful initiation of metamorphosis near stage 35-37, but that for tadpoles on low food any excess energy is allocated to growth.

In contrast to the effects of food increases, decreases in food supply showed no evidence of stage-specific effects on lipid storage (Figure 4-4). Regardless of how long tadpoles experienced high food availability, they metamorphosed with similar fat content. The fat content of tadpoles that experienced food declines was also not different from either the constant high or constant low food controls.

Unlike B. terrestris, P. crucifer showed a significant tendency for tadpoles with high food availability to

metamorphose with significantly higher lipid stores on a percentage basis (Chapter 2). A general model of fat storage in tadpoles must account for the observed patterns in both of these species.

I propose a model of allocation that can explain these results. This model, an extension of Harris's model of proportional allocation (Harris *in press*), emphasizes the importance of size-specific and stage-specific allocation (Figure 4-5). Assimilated energy that is not allocated to maintenance or activity is referred to as production energy, and may be allocated to growth, development, reproduction, or storage. Both body size and developmental stage influence how energy is allocated. Generally body size has the strongest influence, and larger metamorphs have higher fat storage in terms of absolute energy and on a percentage basis, as in P. crucifer (Chapter 2). In some cases, such as B. terrestris tadpoles in the present study, stage-specific effects may dominate allocation.

Central to this model is the relationship between lipid storage and development rates (Figure 4-5 arrow A). This is the relationship originally proposed by Crump (1981), to be a positive correlation. Some support for this proposed relationship is seen in the tendency for B. terrestris tadpoles with high size-adjusted fat storage to metamorphose earlier (Figure 4-2). Under some conditions, however, a negative relationship might be predicted. For example, in extremely ephemeral ponds tadpoles might be predicted to

sacrifice lipid storage in favor of rapid development to avoid death by desiccation.

The influence of developmental stage on the relationship between allocation to growth versus allocation to development (Figure 4-5 arrow B), is central to the dynamic allocation model (Leips and Travis 1994) and the proportional allocation model (Harris *in press*). Developmental stage influences the relationship between growth and storage (Figure 4-5 arrow C), and how this relationship changes when energy income changes. This is seen in the non-allometric responses of size-adjusted lipid reserves to changes in food supply in P. crucifer (Chapter 2), and in stage-specific responses in B. terrestris (Figure 4-4).

Most anurans metamorphose with undifferentiated gonads, and thus allocation to reproduction is inconsequential. In some species, however, gonads differentiate during the final larval stages (Nodzenski et al. 1989, Hsu et al. 1991, Hensley and Anderson *unpublished*), and stage-specific effects on reproductive allocation may become important.

In this model, body-size effects generally dominate the allocation patterns seen in tadpoles, with larger tadpoles metamorphosing with relatively larger fat reserves. Tadpoles raised on high food are predicted to store a greater fraction of their total lipid reserves at earlier developmental stages than do tadpoles on low food, but this stage-specific pattern is overshadowed by body size effects. When tadpoles raised on high and low food levels metamorphose with similar size-

adjusted lipid reserves, such as B. terrestris in this study, stage-specific effects are predicted to be more apparent. In this case, lipid storage might proceed at similar rates, rather than occurring earlier for tadpoles on high food (Figure 4-6A,B).

The model includes a hypothesis that changes in food supply are potentially associated with changes in allocation, and this potential depends on the stage when food supplies change (Figure 4-6C). This hypothesis leads to predictions of stage-specific allocation responses to changes in food availability (Figure 4-6D,E,F). Tadpoles that experience food increases at intermediate stages are predicted to be able to allocate a greater fraction of their energy income to fat storage.

In contrast, tadpoles that experience declines in food supply midway through development might be predicted to metamorphose with significantly reduced fat compared to controls. They may not, however, if most lipid storage occurs at earlier stages when food availabilty is higher. A period on high food in the early part of larval development could result in both rapid growth and a high rate of lipid storage. A decrease in food supply would affect both growth and development, but these tadpoles might experience both the peak in fat storage associated with high food supply, and then the later peak on low food supply. Such stage-specific allocation could result in similar size-adjusted reserves at metamorphosis for tadpole on high, low, and decreasing food

supplies. The final lipid reserves would depend on the actual shape of the stage-specific allocation curves.

The pattern observed in B. terrestris might be the product of programmed stage-specific responses to changes in food supply, or to a gradient of changing plasticity in allocation. One interpretation of the pattern is that food increases at intermediate developmental stages generally result in programmed increases in lipid storage, and that this program is fixed. An alternative interpretation is that the pattern is the product of a gradient in plasticity, and that tadpoles that experience fluctuating food supplies at intermediate stages are more plastic in their allocation response than are tadpoles that experience such changes at earlier or later stages. This ontogenetic change might represent gradual increases and decreases in plasticity, or might be due to abrupt changes between fixed and plastic allocation patterns. Further tests of stage-specific responses are necessary to quantify the changes in plasticity and characterize which developmental stages show plasticity in allocation.

This model of stage-specific allocation is designed to be general, accommodating the patterns seen in P. crucifer (Chapter 2) and in B. terrestris. In neither of these two studies did feeding treatments generate much difference in timing of metamorphosis, in contrast to several previous studies (Wilbur and Collins 1973, Travis 1984, Alford and Harris 1988, Hensley 1990, 1993). Whether the proposed model

is adequate under conditions where feeding treatments strongly affect timing of metamorphosis remains to be seen.

In B. terrestris there is evidence that rapid development is associated with high size-adjusted lipid storage, as suggested by Crump (1981). Under conditions of extreme food limitation or of time constraints on development, however, one might predict a trade-off between rapid development and greater lipid storage. Pfennig (1992) found evidence for such a trade-off between omnivore and carnivore morphs of the spadefoot toad Scaphiopus couchii. Rapid growth and development of carnivore morphs was associated with low fat storage. Omnivores metamorphosed later and smaller, but with absolutely larger fat bodies. Carnivores were more successful in short duration ponds, where developmental timing was constrained; omnivores, however, had higher post-metamorphic survival. The tendency for rapidly growing carnivores to have smaller fat bodies than slowly growing omnivores is not consistent with Crump's (1981) hypothesis, but is accommodated by the model proposed above. Pfennig (1992) indicated that this relationship was part of an evolutionarily stable strategy that allows spadefoots to persist in a desert environment where pond permanence is highly unpredictable. The model proposed above appears adequate for highly specialized tadpoles with polymorphisms that reflect unique adaptations.

The diversity of patterns in larval fat storage seen in P. crucifer, B. terrestris, and S. couchii points to the

importance of studies of energy allocation in complex life cycles. Simple growth-based or size dependent models are common, but may often be inadequate for predicting the dynamics of developmental responses to fluctuating environments. Models for predicting life history transitions such as maturity, metamorphosis, or diapause are stronger if they include stage-specific or age-specific patterns of energy allocation and responses to changing environments.

Table 4-1. Analysis of covariance for $\log_{10}$ lipid storage in B. terrestris tadpoles. Type I sums of squares are used to test sequential hypotheses explained in the text. Probabilities significant at the $\alpha = 0.05$ level are marked with *.

Source	df	Type I SS	MS	F	P
dry mass	1	1.09×10^{-5}	1.09×10^{-5}	371.26	.0001*
family	1	4.41×10^{-7}	4.41×10^{-7}	15.04	.0002*
treatment	7	3.78×10^{-7}	5.40×10^{-8}	1.84	.0852
day of metamorphosis	1	4.43×10^{-7}	4.43×10^{-7}	15.10	.0002*
Residual	127	3.73×10^{-6}	2.94×10^{-8}		

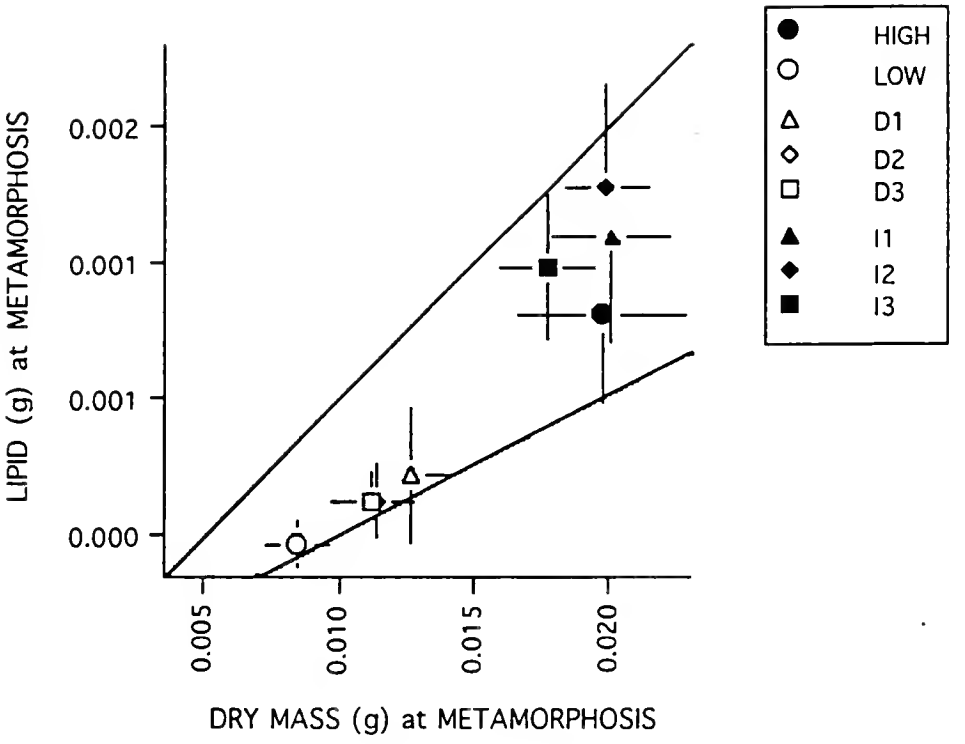

Figure 4-1. Mean mass of stored lipids versus mass at metamorphosis for each treatment group. For clarity, only one side of some 95% confidence intervals is shown. The upper diagonal represents a 10% fat content, by mass; the lower diagonal represents 5% fat.

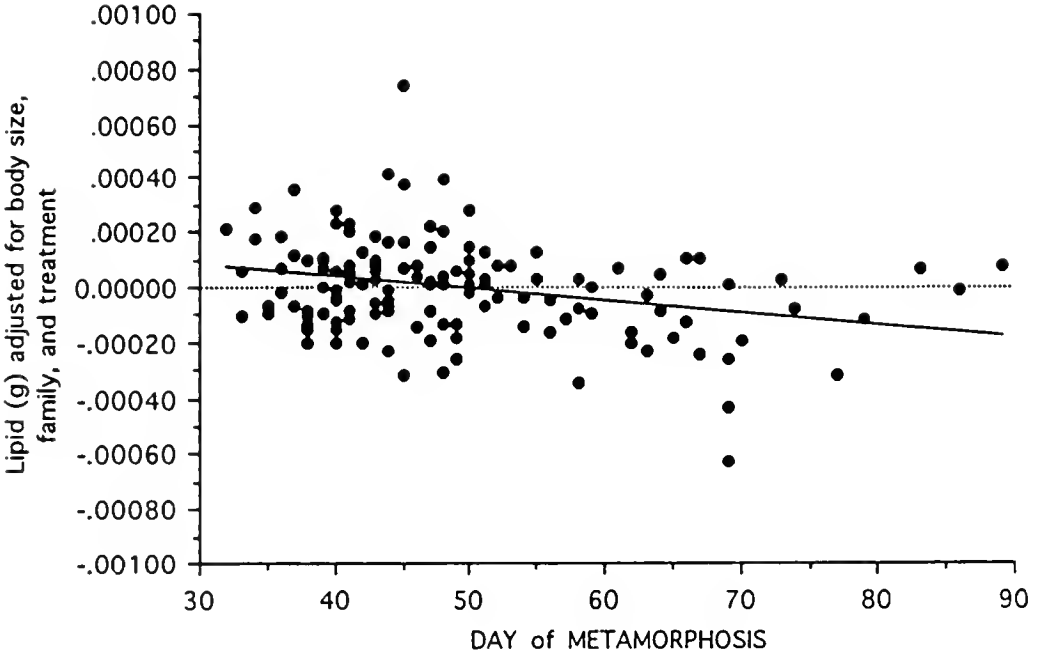

Figure 4-2 Regression plot for the relationship between adjusted lipid storage and day of metamorphosis. This plot represents the final step in the analysis in Table 4-1. After correcting for the effects of body size, sibship, and feeding treatment, there is a decline in allocation to lipids as length of the larval period increases. The slope of the regression (-5.39×10^{-6}) is significantly different from zero ($P = 0.0002$) but the relationship is not strong ($R^2 = 0.087$).

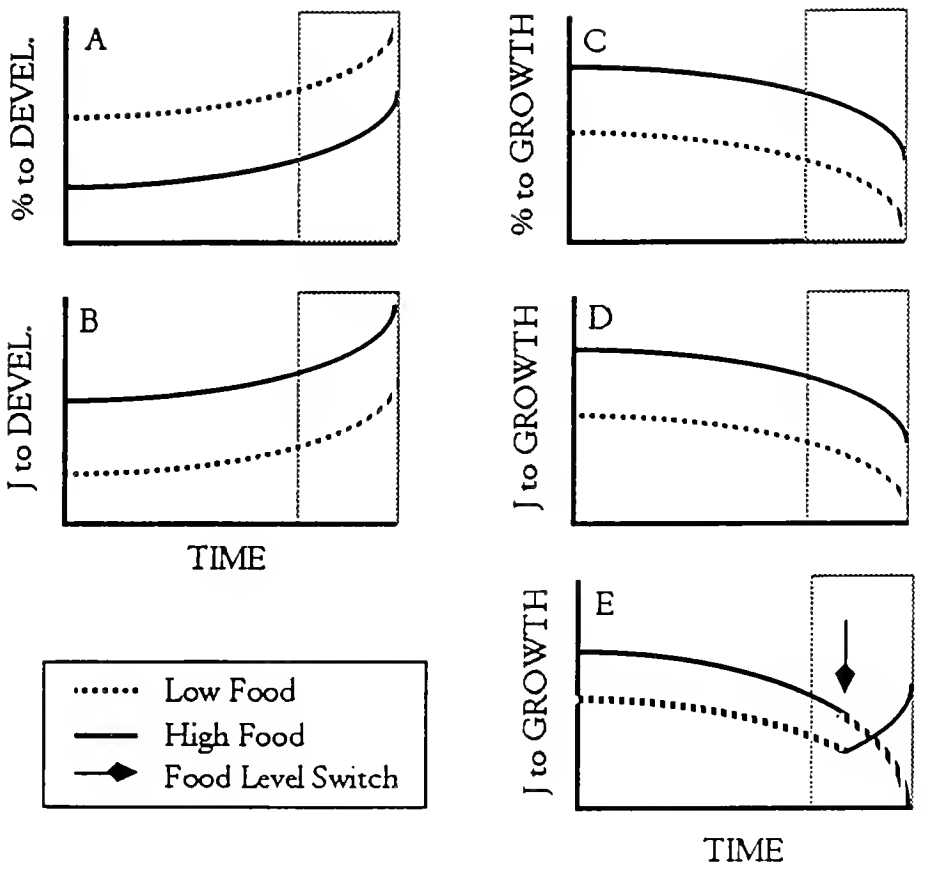

Figure 4-3. A graphical presentation of a model for dynamic allocation of energy to growth and development in tadpoles (modified from Harris *in press*). Each rectangle indicates the period when development rate and developmental expenditure are fixed and insensitive to changes in food supply.
A. The proportion of energy allocated to development.
B. Absolute energy (Joules) allocated to development.
C. Proportional allocation to growth.
D. Absolute allocation to growth.
E. Absolute allocation to growth when food availability changes (arrow) after development rate is fixed.

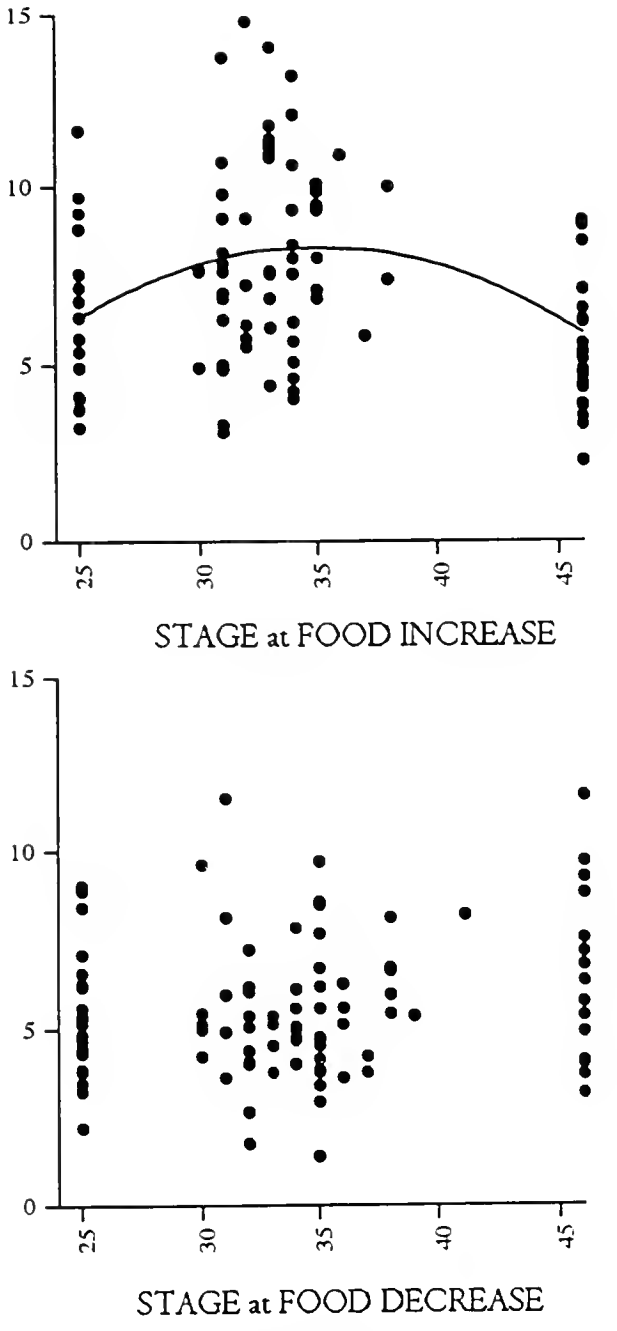

Figure 4-4. The effect of developmental stage at which food supply changed on lipid reserves at metamorphosis. The low food control group is equivalent to a food decrease at stage 25 or a food increase at stage 46 (i.e., never). The high food control is equivalent to an increase in food at stage 25 or a decrease at 46. Stage when food increased affected percent lipid at metamorphosis, and both linear and quadratic terms were significant ($P < 0.001$). Stage at food decrease had no effect on lipid reserve at metamorphosis.

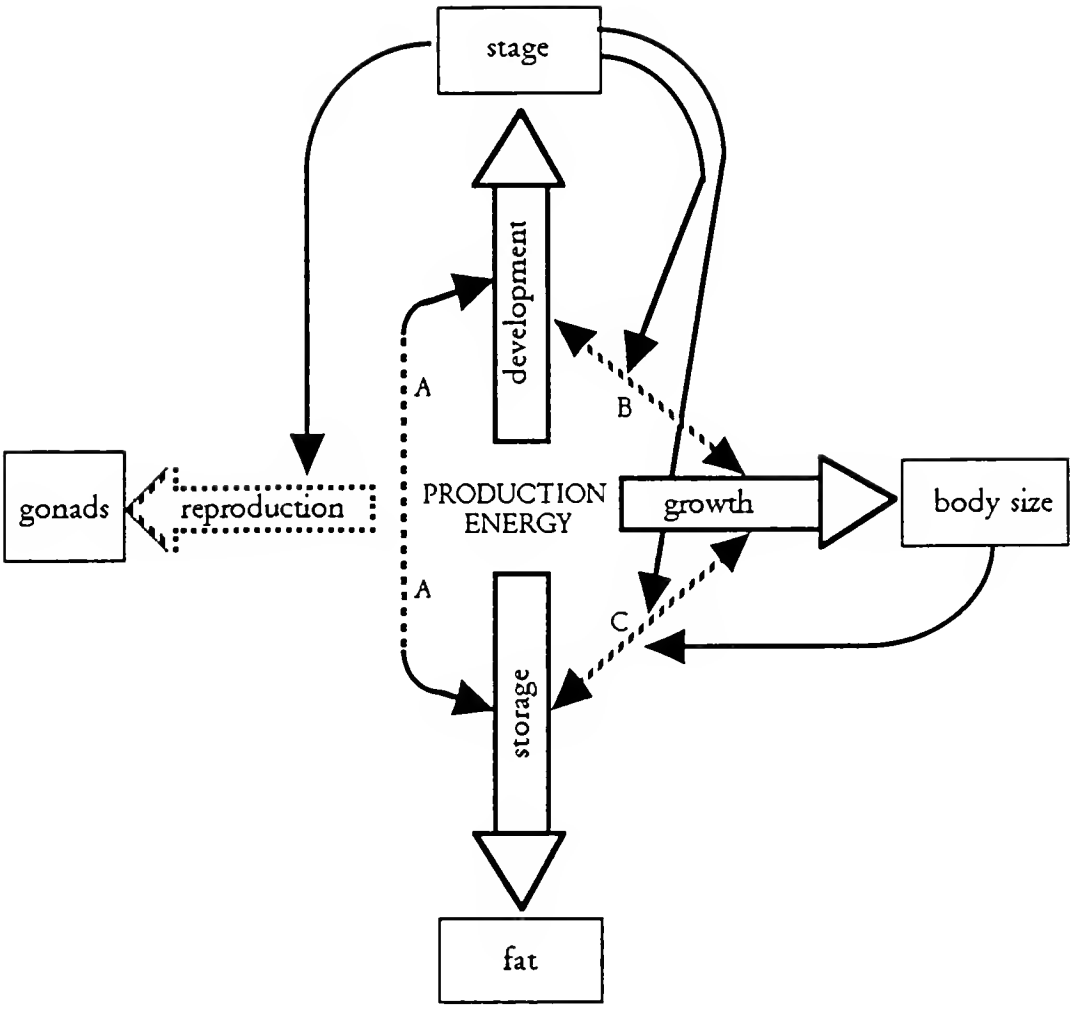

Figure 4-5. A graphical presentation of allocation in tadpoles. Energy transfer functions are shown by wide arrows and energy sinks by boxes. Dashed arrows indicate relationships between energy transfer functions.
A. The relationship between development and energy storage was first proposed by Crump (1981).
B. The trade-off between growth and development is central to the models of Leips and Travis (1994) and Harris (*in press*).
C. Growth and storage are competing functions, but the trade-off is often not detected (van Noordwijk and de Jong 1986, Houle 1991).
Solid black arrows show the influence of body size (allometry) and developmental stage on energy transfers; either may dominate the relationship between growth and storage. For some species, allocation to reproduction may begin in later larval stages.

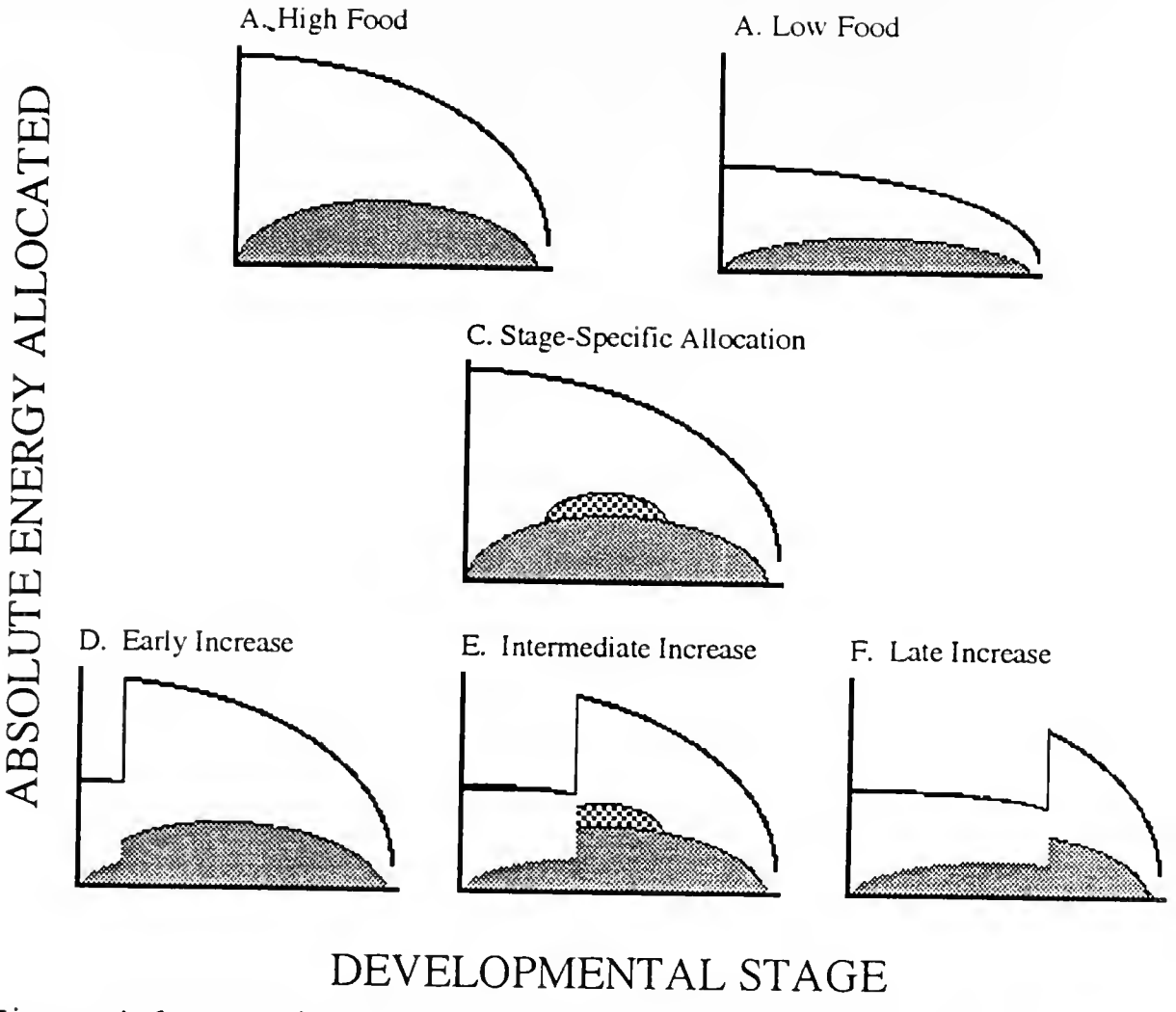

Figure 4-6. In this model, total energy allocated to growth declines over time (Harris *in press*), and is indicated by the area under the upper curve. Allocation to lipid storage is shown as the lower, shaded region. Size-adjusted fat reserves at metamorphosis can be equivalent on high and low food (shaded areas in A and B represent the same proportion of allocation to growth). Panel C presents the hypothesis, that tadpoles at intermediate developmental stages have greater plasticity in allocation. The checkered region indicates the additional lipid storage that can occur when intermediate stage tadpoles experience food increases. Panels D-F are predictions from C. Tadpoles that experience food increases early (D) or late (F) in development, maintain the same proportional allocation as in constant food environments (A,B). A food increase at an intermediate stage (E), when allocation patterns are more plastic, results in proportionally more fat storage (checkered region) To estimate a tadpole's energy allocation requires knowledge of both its current stage and food supply, but also its historical food supply.

CHAPTER 5
STAGE-SPECIFIC ENERGY ALLOCATION IN AN UNPREDICTABLE ENVIRONMENT: EFFECTS OF EARLY POND DRYING ON GROWTH, DEVELOPMENT, AND FAT STORAGE IN *BUFO TERRESTRIS* TADPOLES

Introduction

All organisms face the challenge of allocating assimilated energy among the competing functions of growth, maintenance, and reproduction. Energy allocated to one function is unavailable for the others, but assimilated energy can be stored for future allocation to one of these three competing functions. The influence of energy storage on allocation strategies is poorly understood (Meffe and Snelson 1993) in spite of the fitness consequences of allocation strategies (reviewed in Perrin and Sibly 1993).

Organisms with complex life cycles undergo a morphological metamorphosis, usually accompanied by a habitat shift (Wilbur 1980). An energy reserve may be critical for surviving the metamorphic period (Crump 1981) and dispersal into the new habitat. For amphibian larvae, age and size at metamorphosis are phenotypically plastic traits that respond to environmental conditions. Models have been developed to predict age and size at metamorphosis, but these models do not predict how energy is allocated between growth and

storage when conditions in the environment change unpredictably.

Models have been proposed to explain the phenotypic responses of amphibian larvae to environmental variables such as habitat duration, predation, food supply, and seasonality (reviewed in Alford and Harris 1988, Hensley 1993, Harris *in press*). One model, proposed by Wilbur and Collins (1973), has been central to understanding how larval growth and development respond to environmental variables. The major tenets of the Wilbur-Collins model are that metamorphosis is not possible below a certain minimum body size (b) but is obligate at some maximum size (b + c). Within this size range, metamorphosis is initiated when growth rate falls below some size-dependent threshold (g) (Wilbur and Collins 1973). This model, with some refinements, has withstood several empirical tests (reviewed in Alford and Harris 1988, Hensley 1993, Harris *in press*) and has provided the best framework for understanding the ecology of amphibian larval growth and development.

In a study of the Wilbur-Collins model, Crump (1981) found that tadpoles raised under crowded conditions metamorphosed with less energy per unit body mass than did tadpoles raised at low density. Crump suggested that initiation of metamorphosis may require not simply attaining a minimum body size (b), but also a minimum energy reserve, and that successful models of amphibian metamorphosis should include energy storage in addition to growth and development.

The dynamics of energy allocation in tadpoles have only recently been studied. In a previous study (Chapter 2) I found that fat storage in tadpoles is strongly allometric, but that tadpoles also exhibit phenotypic plasticity in fat storage independent of body size. Fat storage can also be a significant predictor of timing of metamorphosis (Chapter 4), which confirms Crump's (1981) suggestion that models for predicting metamorphosis can be improved when energy allocation is considered.

Previous studies of energy allocation in tadpoles (Chapters 2-4, Leips and Travis (1994)) have been limited, however, to studies of individually raised tadpoles and their responses to changes in food availability. Many other factors such as pond drying, temperature, and predation risk can influence age and size at metamorphosis, and perhaps energy allocation. The dynamics of energy allocation in response to these factors have not been studied.

I proposed a model of energy allocation in tadpoles that emphasizes the importance of allometric and stage-specific allocation to fat storage (Chapter 4). A key element of this model is the relationship between development rate and energy storage rate, which Crump (1981) asserted should be positive. I predicted, however that under conditions where time constraints on development are extreme, such as when ponds dry unpredictably, lipid storage may be traded against rapid development. Pfennig (1992) provided evidence that this

trade-off occurs in spadefoot toads, Scaphiopus couchii, in very ephemeral ponds.

I chose to examine the response of tadpoles to early pond drying because several species are known to accelerate development facultatively in response to habitat desiccation. This developmental acceleration has been demonstrated to have a genetic component and is considered to be adaptive (Newman 1988a, Semlitsch et al. 1990, Newman 1992). The mechanism of this acceleration of development is unknown. One possible factor in this acceleration may be a shift in allocation of energy away from storage and toward increased growth and rapid development.

Two questions are central to this study. First, do tadpoles adjust their energy allocation in response to early pond drying? I predicted that tadpoles from short duration ponds would metamorphose early, with reduced fat storage. Second, do tadpoles from different genetic backgrounds (sibships) respond to early pond drying in different ways? I predicted that sibships that tend to develop more rapidly would be less influenced by short duration ponds than would sibships with slower development.

Methods

Previous work has demonstrated genetic variation for amphibian larval growth and development, including genetic variation for responses to early pond drying (Berven et al.

1979, Newman 1988a, Semlitsch et al. 1990). Because of this genetic variation, I designed the experiment to test the responses of three full sibling families to early pond drying. Response variables were days to metamorphosis, body mass, and mass of lipid stored at metamorphosis.

I used a 3 x 2 factorial experiment in a randomized complete block design with sibship and drying treatment as the factors. The experiment was conducted in 18 cattle watering tanks (1.5 m diameter x 0.6 m tall) in a field at the Savannah River Ecology Laboratory, Aiken, South Carolina. Tanks were arranged in three adjacent rectangular blocks to control for spatial heterogeneity in sunlight, and treatments were assigned randomly within blocks.

Each of the 18 tanks was lined with a flexible plastic liner. Six days prior to the experiment, tanks were each filled with well water to a depth of 50 cm (≈880 L) and stocked with 0.5 kg of dry leaf litter collected from the forest floor adjacent to a nearby temporary pond. Because the leaf litter was dry, it was assumed to be free of aquatic predaceous insects. Tanks were covered with fiberglass screen lids (1.5 mm mesh) to prevent colonization by predaceous aquatic insects and treefrogs. Three days prior to the experiment, tanks were inoculated with equal volumes of a thoroughly mixed plankton suspension drawn from six nearby ponds. On day 3 of the experiment, a second inoculum from two nearby ponds was added. Inoculation with plankton from several sources establishes a diverse community of

microorganisms as a base for a complex food web in each tank (Wilbur 1987).

I collected Bufo terrestris eggs from three clutches in a shallow roadside puddle that had filled two days previously. Single clutches of eggs were identified in the field by the size of the egg mass, distance from other egg masses, and a lack of strings of eggs connecting egg masses. I maintained hatchlings for four days until they reached Gosner developmental stage 25 (Gosner 1960), at which point tadpoles begin feeding. I haphazardly selected tadpoles one at a time from each family. Tadpoles were placed in groups of 10 in the order they were selected. I then randomly combined these small groups to form full-sibling groups of 200. This randomization process reduced any bias in tadpole size or behavior that might have influenced the selection process. I assigned each group of 200 randomly to either a drying or constant-depth tank. The density of 200 tadpoles per tank is a relatively low density and was chosen to ensure large numbers of successful metamorphs from tanks that dried early (Wilbur 1987).

The drying treatment was applied to tanks using the formula from Wilbur (1987):

$$D_j = 1 - (j/t)^a P$$

where D_j is the depth on day j, j is time in days since the start of the experiment, t is the target date for depth = 0, P is the depth at the start of the experiment (50 cm in this study) and a determines the shape of the drying curve (a =

2). For this experiment I set t = 49 days. Depth in drying tanks was reduced by adjusting the angle of a rotating overflow standpipe in each tank. I made depth adjustments in 5 cm increments (days 15, 22, 27, 31, 35, 38) down to a depth of 20 cm (day 38), and then made daily adjustments according to the formula until the tanks dried completely on day 49. Constant-depth tanks ranged in depth from 45 - 50 cm. The experiment continued until all tadpoles in the constant-depth tanks metamorphosed (day 58).

For this experiment I defined metamorphosis behaviorally, rather than using a specific developmental stage. Tadpoles that voluntarily left the water were considered metamorphs, as were tadpoles that had external front legs (stage ≥ 42, Gosner 1960) and attempted to evade capture by swimming on the surface using their back legs for propulsion rather than diving or using caudal propulsion. This behavioral definition thus included Gosner stages 42-46, and eliminated the need to disturb the tank water and substrate to search for metamorphs.

Metamorphs were collected daily, and date of metamorphosis was recorded for each individual. Most metamorphs were released at the site where the eggs were collected, but subsamples were retained each day for size and lipid measurements. Metamorphs used in lipid analysis represent a non-random subsample of each tank with respect to age at metamorphosis because the final distributions of metamorphs were unknown when animals were selected. For any

given day, however, toads retained for lipid analysis were either a random sample from each tank, or in some cases included all of that day's metamorphs from a tank.

Lipid extractions for individual tadpoles were conducted using methods modified from Reznick and Braun (1987). Whole frozen tadpole carcasses were dried in thrice tared $^1/_2$ dram glass shell vials at 55°C. Dried tadpoles were stored over $CaSO_4$ desiccant and weighed three times to the nearest 0.1 mg. Lipids were extracted by soaking each tadpole in room-temperature petroleum ether, which preferentially dissolves non-polar storage lipids (triglycerides and free fatty acids) (D.L Schultz *personal communication*, Hensley *unpublished*). At hourly intervals ether was pipetted off and replaced. Previous work indicated that 7 one-hour soaks was in excess of that needed to extract the tadpoles to a constant mass (Hensley *unpublished*). After extraction, tadpoles were again oven dried at 55°C and weighed three times. Mean values of tare mass and mass before and after extraction were used to calculate tadpole total dry mass and total mass of lipid extracted.

Statistical Analysis

Treatment and sibship effects on days to metamorphosis, size at metamorphosis (dry mass), and lipid storage (mass extracted) were tested with multivariate and univariate analyses of variance (MANOVA, ANOVA). Response variables

were $\log_{10}(x+1)$ transformed to meet the assumption of homogeneity of variance. I examined differences among families using Scheffe's tests, and within-family responses using planned contrasts (df = 1). All statistics were calculated using SuperANOVA® 1.1 software (Abacus Concepts Inc. 1989).

Experiments similar to this one have been analyzed in two ways, depending on the focus of the studies and the concerns of committing Type I and Type II errors. Tadpoles within a cattle tank are not statistically independent, because competition within a population can generate both negative and positive covariances of age and size at metamorphosis (Collins 1979, Wilbur 1987). One approach to this problem is to test treatment effects on mean values of each independent tank. Using the (treatment x block) mean square as the denominator in F-tests is tantamount to testing tank means (Wilbur 1987). This analysis is conservative and protects against incorrectly rejecting the hypothesis of no treatment effect.

Testing tank means, however, may obscure significant variation among individuals in their allocation patterns (Bernardo 1994) and thus lead to failure to reject the hypothesis of no treatment effect when, in fact, early pond drying alters energy allocation. When allocation patterns of individual animals are the focus of the study, treating individuals as the unit of observation, rather than tank means, is essential (Bernardo 1994). Given the advantages

and disadvantages of these two approaches, and the different interpretations that arise from them, I conducted both an analysis of tank means and an analysis of individual allocation. I used multivariate analysis of variance (MANOVA) and its component ANOVAs to test for treatment effects on tank means. I then used analysis of covariance (ANCOVA) to test for treatment effects on size-adjusted lipid storage among individual tadpoles.

Results

No metamorphs were present in the tanks on day 32 of the experiment. On day 35 both live and dead metamorphs were present in 17 tanks, suggesting that metamorphosis probably began 1-2 days earlier but that some metamorphs did not successfully climb the plastic tank liners, and drowned. To alleviate this problem, two strips of fiberglass mesh (ca. 20 x 60 cm) were suspended in each tank from stiff wires attached across the tank. These mesh strips were perpendicular to and flush with the tank walls so that metamorphs swimming along the tank wall would encounter a mesh strip and be able to climb up. Complete counts of dead metamorphs for days 33-35 were not possible without disturbing the leaf litter substrate. Because of the mortality of metamorphs from days 33 to 35, subsampling for lipid analysis did not begin until day 36. A total of 1582 metamorphs were collected from day 36 to the end of the

experiment, and a subsample (N = 558) was retained for lipid extraction.

Analysis of Days to Metamorphosis (Tank Means)

Family and block effects on age at metamorphosis were significant, but the drying treatment did not have a significant effect on the length of the larval period (Table 5-1). Family 3 took significantly longer to complete development than Families 1 and 2 (Scheffe's test, P = 0.001, P = 0.029, respectively). Family 3 developed significantly faster (P = 0.0001) in drying tanks than in constant-depth tanks, but Families 1 and 2 did not (Figure 5-1). Block effects and their interactions were highly significant, indicating high spatial variation among tank means.

Analysis of Size and Lipid Storage (Tank Means)

Family effects significantly influenced the multivariate response vector of day of metamorphosis, lipid storage, and mass (Figure 5-2, Table 5-2). The differences in reaction norms for days to metamorphosis in Figures 5-1 and 5-2A reflect subsampling. There were insufficient degrees of freedom to calculate MANOVA statistics for the drying treatment effect using the (treatment x block) error term, so analysis of the treatment effect on tank means is limited to univariate procedures, below. The block effect and all interactions containing the block term were significant

(Table 5-2), indicating strong spatial heterogeneity in the experiment.

Univariate analysis (Table 5-3) showed that drying treatment and sibship did not significantly affect mean lipid storage among the tanks (Figure 5-2B), but block and block interactions indicated spatial influence on lipid storage.

Timing of metamorphosis in the subsampled tadpoles showed the same pattern of significance (Table 5-3) as seen when all metamorphs were included (Table 5-1).

Drying treatment had a marginal effect on size at metamorphosis (Table 5-3, Figure 5-2C). Contrasts between short and long duration ponds showed that drying treatment resulted in smaller metamorphs in Families 1 and 3 ($P = 0.031$, $P = 0.013$, respectively), but not in Family 2.

Analysis of Size and Lipid Storage (Individuals)

In a preliminary analysis the block effect was not significant, so it was pooled with the error term for the final analysis. The analysis of covariance (ANCOVA) showed that sibships did not differ in the relationship between body size and lipid storage. Allocation to lipid storage increased with body size, but this relationship was significantly affected by drying treatment (Figure 5-3). For Families 2 and 3 tadpoles tended to metamorphose from drying tanks with higher size-adjusted lipid reserves than tadpoles from constant-depth tanks.

Discussion

When the results of this experiment are examined at the level of mean response of populations in each cattle tank (Tables 5-1,2,3), there is little to suggest that energy allocation patterns are significantly affected by early pond drying. Full-sibling families differed in their development rates, size at metamorphosis, and in how these two variables were influenced by early pond drying. Mean values of lipid storage, however, were not significantly different among families or between drying and constant tanks. Spatial variation among tanks had a strong influence on phenotypes, including mean lipid storage. Both sibship and drying appeared to influence lipid storage only through interactions with the spatial blocks (Table 5-3). The relationship among these factors is unclear when analysis is limited to mean responses of cattle tanks.

A more detailed understanding of energy allocation patterns is evident in the ANCOVA used to examine size-adjusted lipid storage among individuals (Table 5-4). Body size (dry mass) is highly correlated with mass of stored lipid. After correcting for size effects, differences among families are not significant, but drying treatment explains a significant fraction of the remaining variation in lipid storage. The significant interaction of drying treatment and body size indicates that the regression slopes (Figure 5-3) are significantly different for drying versus constant tanks.

I predicted that tadpoles induced by pond drying to metamorphose early would do so with less lipid per unit size than tadpoles from constant tanks, as allocation to storage would be reduced in favor of more rapid growth and development. In Figure 5-3, however, it is evident that the predicted trend did not occur; in Families 2 and 3 large tadpoles from drying tanks tended to have more fat per unit size than tadpoles from constant tanks.

An explanation for this allocation pattern can be found in the relationship of growth rates and development rates. In a previous study (Chapter 4) I showed that B. terrestris tadpoles within a sibship vary in growth and development rates, and in lipid storage, but there is no evidence for a trade-off among these variables. Tadpoles with high intrinsic growth rates develop rapidly and have large lipid reserves at metamorphosis. The lack of a trade-off between allocation to lipids and greater growth or development is explained by variance in the ability of individuals to gather and assimilate energy (van Noordwijk and de Jong 1986, Houle 1991). Tadpoles with high energy acquisition rates will grow, develop, and store fat more rapidly than siblings that acquire energy more slowly. Therefore, when a pond dries early the largest tadpoles present would be expected to have the highest rates of fat storage in the population.

Given this variance in performance, when ponds dry early less competitive tadpoles may allocate less energy to fat storage, as predicted. Meanwhile, the largest and most

competitive tadpoles in a pond may maintain high rates of energy storage. Under these conditions, the mean lipid storage for the population may be unaffected, but the relationship of lipid storage to body size could be much steeper (Figure 5-3).

An additional factor that may play a role in shaping the population's response to pond drying is the induction of early metamorphosis. Evidence suggests that in some species tadpoles do not store lipids at a constant rate during development (Chapter 3). For example, in Rana catesbeiana tadpoles, most lipid storage occurs by developmental stage 35, but substantial growth continues through later stages (Figure 5-4, Hensley and Anderson *unpublished*). If the same is true for B. terrestris tadpoles, then early metamorphosis induced by pond drying may truncate growth for tadpoles that have already attained large lipid reserves. Thus while small, less competitive tadpoles may respond to pond drying by reducing allocation to lipids, large tadpoles may metamorphose early, with high mass-specific lipid reserves. This would account for the steeper slope of the relationship between body size and lipid storage seen in tanks that dried early (Figure 5-3, Families 2 and 3).

This pattern of stage-specific lipid storage and a dynamic relationship between development rate and lipid storage rate is consistent with a model of dynamic allocation (Chapter 4). According to that model, developmental stage influences both the relationship of energy allocation to

growth versus development and the allometry of size-specific lipid storage. Generally allometric effects dominate, but stage-specific effects are often manifested in fluctuating environments. The change in allometry of lipid storage between early-drying ponds and constant ponds supports the contention that maximal lipid storage occurs at earlier developmental stages for rapidly growing tadpoles.

The present study reinforces the idea that energy allocation in tadpoles is a dynamic process that is influenced by genetic constitution (sibship effects) and by abiotic conditions. Although the role of genetic variation in phenotypic plasticity of tadpole growth and development has been studied (Berven et al. 1979, Newman 1988a, Semlitsch et al. 1990), a complete understanding of plasticity in energy allocation will require the use of quantitative genetics in future studies. Nevertheless, certain among-family patterns are evident in this study. Sibships differed in development rate, and in how they responded to early tank drying (Figure 5-1). Early pond drying affected body size and development rate for Family 3, which had the slowest mean development. Families 1 and 2 maintained rapid development rates in both drying and constant tanks, but Family 1 did so in the drying tanks at the expense of body size.

These among-family differences can be interpreted in terms of models for predicting metamorphosis (Wilbur and Collins 1973, Hensley 1993) and dynamic allocation priorities (Leips and Travis 1994). Because the families differed in

overall development rate, the effects of drying treatments can be thought of as occurring at earlier developmental stages for Family 3 than for Families 1 and 2. As tadpoles develop, their sensitivity to environmental factors changes and development rate becomes fixed (Hensley 1993). Tadpoles in Family 3 may have experienced the effects of pond drying while development rates were plastic. Development rates of Families 1 and 2 may not have been affected by pond drying because drying occurred at later developmental stages. After development rates become fixed, plasticity in growth rates persists (Hensley 1993); this pattern is evident in the response of Family 1, which metamorphosed smaller in drying tanks, but not significantly earlier.

This ontogenetic change in plasticity has been characterized as a change in allocation priorities (Leips and Travis 1994), with tadpoles preferentially allocating resources to rapid development in the early stages of larval period, but with development becoming a decreasing priority over time, and growth taking precedence (but see Chapter 3). Lipid storage can be considered as a third allocation priority that such models should consider. If a minimum energy reserve is a prerequisite for successful metamorphosis (Crump 1981), then lipid storage should be a high allocation priority until this minimum is achieved, after which it would be expected to be subordinate to allocation to growth, depending on the relative benefits of large size at metamorphosis and larger lipid reserves. A tendency for most

lipid storage to occur by approximately stage 30 (Figure 5-4) is consistent with this model of allocation and with high lipid reserves in tadpoles from tanks that dry early.

One other study has suggested that phenotypic plasticity in energy allocation may be important to successful metamorphosis under varying environmental conditions. Pfennig (1992) studied spadefoot toad (Scaphiopus multiplicatus) tadpoles that occur as either slow-developing omnivores or fast-developing carnivores. The inducible carnivore phenotype had the advantages of rapid development and large body size at metamorphosis, but omnivores metamorphosed with large fat bodies that appeared to contribute to their higher post-metamorphic survival. Pfennig argued that this difference in post-metamorphic survival explains the maintenance of the polymorphism as an evolutionarily stable strategy. The polymorphism occurs only in some species of Scaphiopus, so generalizations about energy allocation to species without polymorphic tadpoles are not possible. In general, however, one would predict that in unfavorable growth conditions, tadpoles will store less reserve energy per unit body size, allocating a greater fraction to growth and rapid development. In these spadefoots there was a clear trade-off between size and lipid storage that is not seen in Bufo terrestris. Perhaps selection in extremely ephemeral ponds has reduced the variance in energy acquisition among individual Scaphiopus,

and this low variance permits the predicted trade-off to be manifested.

The three families of B. terrestris in the present study represent points on a continuum of possible responses to early pond drying. Because the 49-day drying treatment affected timing of metamorphosis for Family 3 only, generalizations about the effects of induced developmental acceleration must be made with caution. An empirical study with shorter pond durations would be predicted to generate greater effects on developmental timing and greater changes in the size-specific reaction norms for lipid storage for all families. A comprehensive study of this relationship would require pond durations that span the range of responses for all sibships in the study. These would include the shortest pond duration that could result in successful metamorphosis, intermediate durations that cover the periods of changing sensitivity, and ponds that never dry. An adequately replicated factorial experiment with several sibships, realistic tadpole densities, and realistic pond sizes, would probably be limited by the number of eggs in a clutch.

In this study I found evidence that energy allocation in tadpoles responds to unpredictable changes in the environment. I speculate that this is in part due to patterns of energy allocation during ontogeny, but it may also have an adaptive component. In my study early pond drying did not significantly influence the mean lipid storage in populations, but did affect the distribution of lipid

storage among individuals. The significance of these differences for survival to maturity and ultimate reproductive success are unknown. If flexible allocation enhances the fitness of tadpoles over the range of environments they encounter, and results in higher fitness than would a single fixed allocation pattern, it would then be considered adaptive. Whether it is adaptive or not, its implications for population dynamics warrant further study.

Table 5-1. ANOVA of days to metamorphosis for all metamorphs (* = P < 0.05). F-tests are tests of tank means, calculated using the interaction with the block term as the denominator.

Source	df	SS	MS	F	P	Error Term
A Drying	1	.012	.012	.636	.5089	A x C
B Family	2	.257	.128	21.625	.0072*	B x C
C Block	2	.080	.040	20.186	.0001*	Residual
A x B	2	.054	.027	1.512	.3243	A x B x C
A x C	2	.037	.018	9.231	.0001*	Residual
B x C	4	.024	.006	2.989	.0180*	Residual
A x B x C	4	.071	.018	8.932	.0001*	Residual
Residual	1564	3.108	.002			

Table 5-2. MANOVA of treatment effects on days to metamorphosis, dry weight and lipid storage for subsampled tadpoles (* = P < 0.05). F-tests are tests of tank means, calculated using the interaction with the block term as the denominator.

Source	Wilks' λ	Num df	Den df	F	P	Error Term
A Drying	(insufficient df for test)					A x C
B Family	.008	6	4	6.714	.0433*	B x C
C Block	.822	6	1084	18.590	.0001*	Residual
A x B	.076	6	4	1.744	.3071	A x B x C
A x C	.975	6	1084	2.339	.0300*	Residual
B x C	.893	12	1434.3	5.237	.0001*	Residual
A x B x C	.879	12	1434.3	5.946	.0001*	Residual

Table 5-3. P values for univariate ANOVAs of treatment effects for subsampled tadpoles (* = P < 0.05, but note marginal significance of some effects). F-tests are tests of tank means, calculated using the interaction with the block term as the denominator.

Source	df	days to metamorphosis	dry mass	Lipid storage	Error Term
A Drying	1	.2073	.0535	.2857	A x C
B Family	2	.0021*	.0604	.1481	B x C
C Block	2	.0003*	.0001*	.0003*	Residual
A x B	2	.5908	.2361	.6349	A x B x C
A x C	2	.3317	.2936	.0257*	Residual
B x C	4	.6544	.0001*	.0154*	Residual
A x B x C	4	.0004*	.4371	.0003*	Residual
Residual	540				

Table 5-4. ANCOVA of treatment effects on lipid storage, adjusted for body size (dry mass). (* = P < 0.05).

Source	df	SS	MS	F	P
A Drying	1	1.90×10^{-7}	1.90×10^{-7}	4.815	.0286*
B Family	2	1.21×10^{-7}	6.03×10^{-8}	1.528	.2179
C Dry Mass	1	9.45×10^{-6}	9.45×10^{-6}	239.448	.0001*
A x C	1	2.60×10^{-7}	2.60×10^{-7}	6.573	.0106*
B x C	2	1.62×10^{-7}	8.09×10^{-8}	2.051	.1296
Residual	550	2.17×10^{-5}	3.95×10^{-8}		

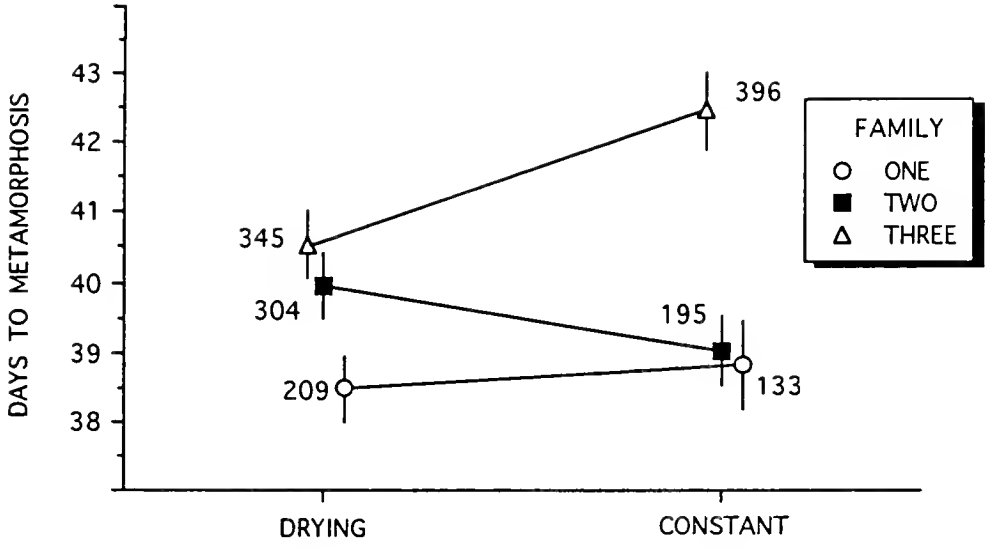

Figure 5-1. Reaction norms for days to metamorphosis for each full sibling family. Error bars are 95% confidence intervals. Sample sizes are shown next to each point.

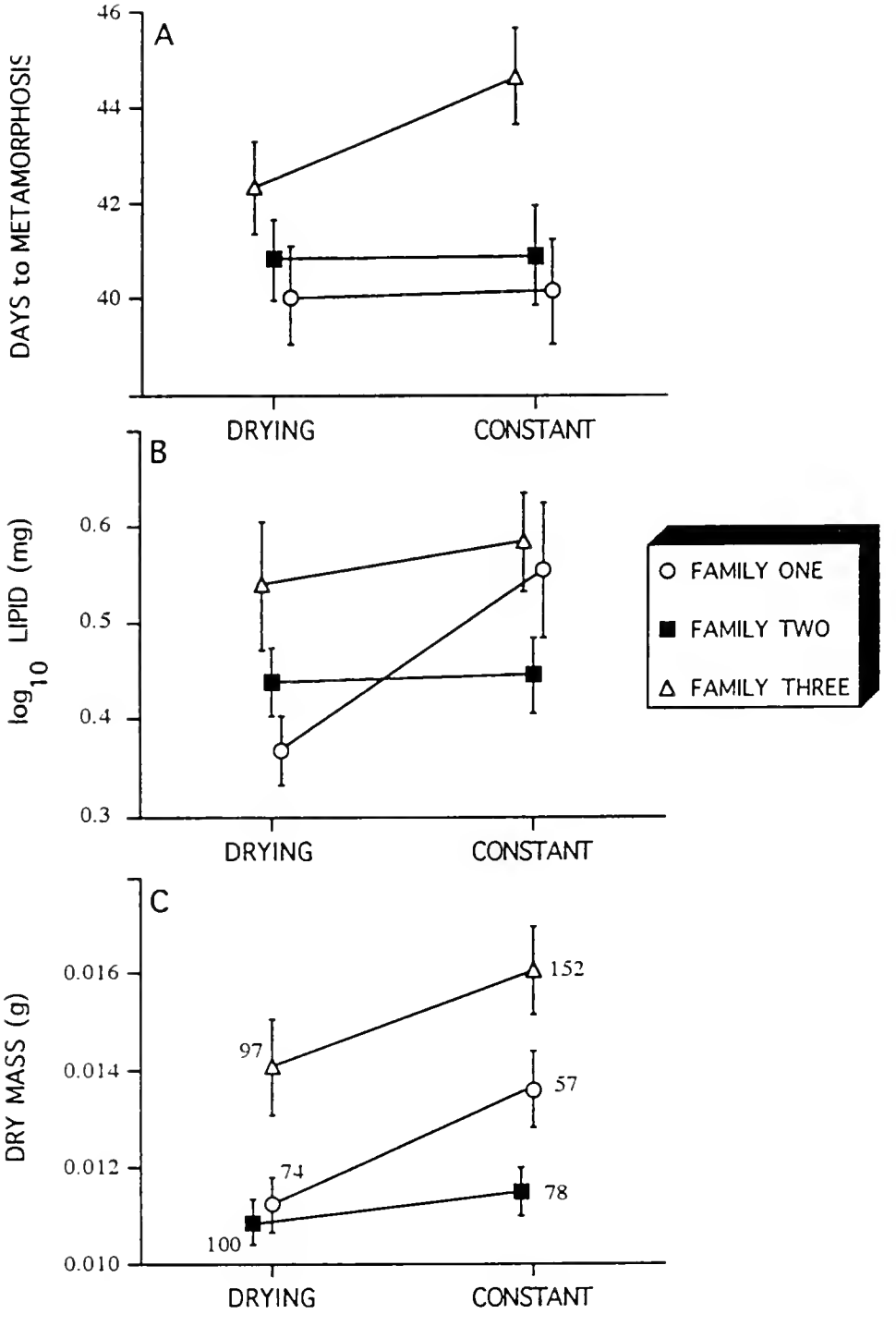

Figure 5-2. Reaction norms for metamorphs subsampled from each full-sibling family. Error bars are 95% confidence intervals, and sample sizes are given in graph C. A) days to metamorphosis B) $\log_{10}$ lipid(mg), and C) dry mass (g)

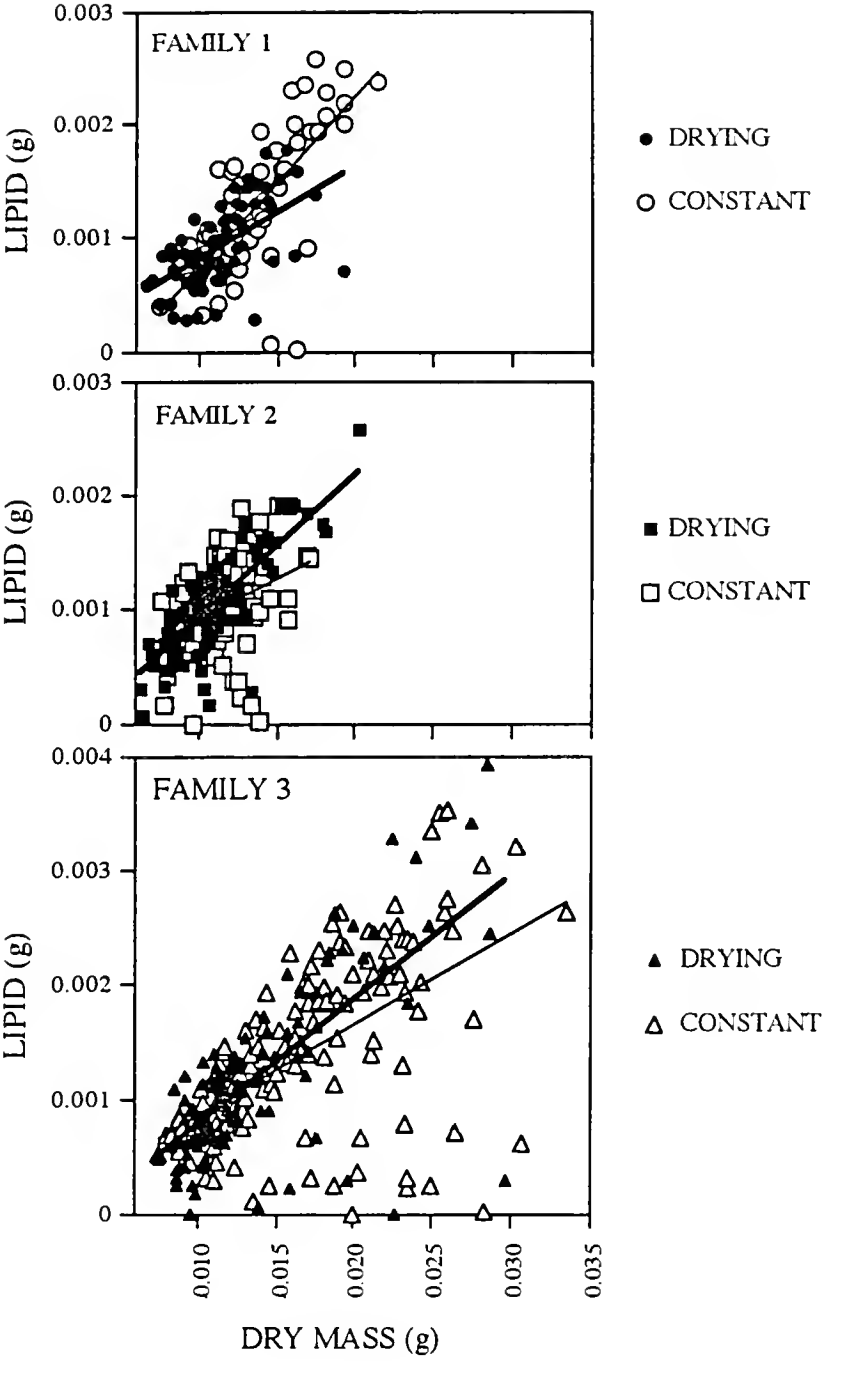

Figure 5-3. Allometry of lipid storage for B. terrestris tadpoles. Solid symbols and bold lines indicate tanks that dried in 49 days; open symbols and fine lines represent constant-depth tanks. Graphs are plotted to the same scale.

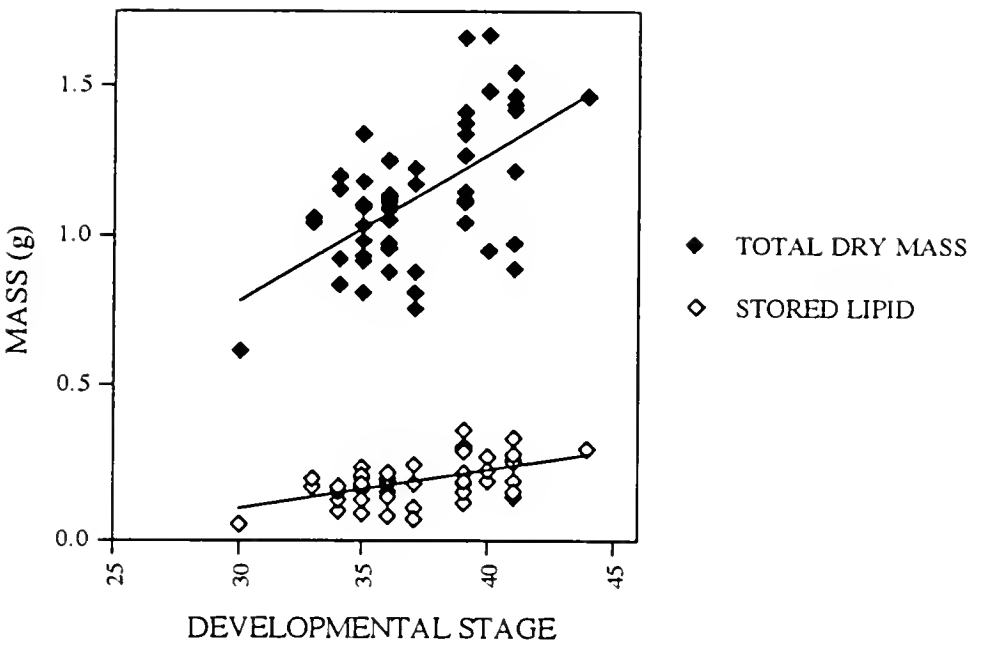

Figure 5-4. Body size (dry mass) and lipid reserves of Rana catesbeiana tadpoles from a single pond. Lipid storage in later developmental stages is relatively slow, while growth is rapid.

CHAPTER 6
SUMMARY AND PROSPECTUS

Summary

How do organisms respond to unpredictable changes in their environment that affect individual fitness? Among amphibian larvae, growth, energy storage, and the timing of metamorphosis are extremely variable and potentially have serious consequences in terms of individual fitness. Because growth, development, and storage are functions that compete for a tadpole's energy income, energy allocation patterns may be significantly affected by environmental variables. Understanding how tadpoles allocate energy in variable environments is a necessary step toward understanding the adaptive significance of complex life cycles.

I began by testing whether fat storage is entirely allometric or is independent of tadpole size. If the effects of environmental variation on fat storage can be explained wholly by effects on body size, then consideration of fat storage in size-related models of metamorphosis is redundant. Because body size is easily and nondestructively measured, it would be preferred over lipid storage if they are equivalent predictors. I experimentally tested the plasticity of lipid storage in *Pseudacris crucifer* tadpoles and found that

changes in food availability affected fat storage independently of effects on body size. I concluded that energy storage is, in fact, worthy of consideration in ecological models of amphibian metamorphosis.

I next examined growth and developmental trajectories of Bufo terrestris tadpoles in the context of ontogenetic changes in energy allocation priorities. I found developmental trajectories to be more informative about changes in plasticity than simply considering the timing of metamorphosis. Developmental trajectories supported the hypothesis that there is a dynamic change over developmental time in the relationship between growth and development. Growth and developmental trajectories were consistent with predictions of the Wilbur-Collins model of metamorphosis (Wilbur and Collins 1973) and showed some support for the dynamic allocation model (Leips and Travis 1994). In general, the two models are complementary, but where they differ the data support the Wilbur-Collins model.

The dynamic allocation model has recently been extended to distinguish between absolute allocation of energy and proportional allocation (Harris *in press*). I examined lipid storage in B. terrestris tadpoles from the above experiment in light of energy allocation models. Neither of the energy allocation models considers energy storage. My results with B. terrestris indicate that stage of development can significantly affect energy allocation and its responses to fluctuations in environmental conditions. I propose a model

of stage-specific allocation to accommodate the patterns of energy allocation seen in B. terrestris and in P. crucifer.

One of the predictions of the model of stage-specific allocation is that tadpoles in rapidly deteriorating environments will respond with increased allocation to development at the expense of energy storage. I tested this prediction experimentally using artificial ponds that dried rapidly versus ponds that remained full. I detected no mean difference in fat storage in B. terrestris tadpoles from drying versus constant ponds. The allometry of fat storage was affected, however; in drying ponds tadpoles tended to have greater fat storage per unit mass than in constant ponds. This increased size-adjusted fat storage was evident in the families that developed most slowly. I interpreted these changes in allometric responses as evidence of stage-specific energy allocation patterns. Thus, although the prediction of reduced fat storage was not supported at the population level, there is evidence from individual variation within populations to support the model.

Prospectus

Several avenues of investigation remain for characterizing phenotypic plasticity in tadpole metamorphosis and its ecological and adaptive significance. From an ecological perspective, the relationships among growth, development, and energy storage are complex. The model I

proposed suggests that in some conditions allometric effects will dominate energy allocation, but in other conditions stage-specific effects may have significant influence. This model raises many questions. Under what conditions are stage-specific effects important, relative to body-size effects? Do species vary in the magnitude or timing of stage-sensitive energy allocation?

In my studies, changes in food supply and changes in pond duration were the only factors considered, and only two species were investigated. Other factors such as predation and competition (reviewed in Alford *in press*), and temperature (Wilbur and Collins 1973, Smith-Gill and Berven 1979, Pandian and Marian 1985) are known to influence tadpole growth and development rates. How variations in these factors might affect energy allocation in various species remains to be seen.

For example, Skelly and Werner (1990) found that tadpoles of Bufo americanus responded to the presence of predatory dragonfly naiads by reducing foraging activity. This led to reduced growth and smaller size at metamorphosis, but did not affect timing of metamorphosis (Skelly and Werner 1990). Under threat of predation tadpoles might be predicted to reduce allocation to fat storage in favor of growth and development. The accuracy of this prediction will depend, however, on how tadpoles respond to the threat of predation. Tadpoles of the gray treefrog, Hyla chrysoscelis, respond to the chemical cues from dragonfly predators with an induced

anti-predator morphology and accelerated growth (McCollum 1993). Tadpoles exposed to dragonfly predation on conspecifics develop deeper tail fins with dark mottled color patterns. This inducible defense increases tadpole sprint speed and maneuverability, and ultimately increases survivorship (McCollum 1993). Preliminary evidence suggests that the induced morphology and accelerated growth are not accompanied by reduced lipid storage (McCollum and Hensley, *unpublished data*). It is possible that species that rely on mobility and maneuverability to escape predation may not suffer the same energetic costs as tadpoles that rely on reduced activity and crypsis to avoid predation.

In addition to studying the dynamics of energy allocation in relation to environmental variation in the aquatic phase, studies of the effects of larval ecology on postmetamorphic juveniles are much needed. Previous work has demonstrated that age and size at metamorphosis can significantly affect individual fitness by influencing postmetamorphic survival (Berven 1990, Pfennig 1992) or age and size at first reproduction (Collins 1979, Smith 1987, Berven 1990). Larval lipid storage may contribute to post-metamorphic survival in spadefoot toads (Pfennig 1992), but whether this is a general phenomenon is unknown. The biggest limitation on studies of the post-metamorphic effects of larval lipid storage is the lack of a non-destructive means of measuring lipid reserves in very small animals. Advances in the use of techniques to estimate lipid storage

nondestructively such as total body electrical conductivity (Walsberg 1988) may eventually provide a means to conduct such studies.

Ecological variation in energy allocation may be adaptive. Recent work has emphasized the importance of characterizing the underlying genetics of plasticity to determine whether plasticity is adaptive (Newman 1988a). Current evidence suggests that plasticity in growth and development of amphibian larvae is heritable and adaptive (Newman 1988a). Determining whether plasticity in energy allocation is truly adaptive will require the use of quantitative genetics to examine genetic variance for plasticity in allocation.

Further studies of energy allocation with respect to predation, temperature changes, and competition will help characterize species-specific patterns of energy allocation. Studies of postmetamorphic consequences of allocation patterns will help clarify the potential adaptive significance of variation in lipid storage. Studies of the quantitative genetics of plasticity in fat storage will determine whether such plasticity is truly adaptive. Ultimately such studies will improve our understanding of how organisms allocate resources to deal with environmental unpredictability.

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BIOGRAPHICAL SKETCH

Frank Hensley was born in 1965, in Colorado, and grew up in Nebraska. He received his B.S. degree in biology from Baylor University in Waco, Texas, in 1987. After graduating from Baylor, Frank and his wife, Adele moved to Florida to pursue graduate degrees in biology. In 1989 Frank had the dubious privilege of seeing the last endangered Costa Rican golden toad, Bufo periglenes. Since that day there have been no documented sightings of the golden toad, but the faithful still have hope.

Frank received his M.S. degree in zoology from the University of Florida in 1990. Frank conducted his doctoral research at the Savannah River Ecology Laboratory, located on the Savannah River Site in Aiken, South Carolina. This was an ironic twist of fate because Frank's father and grandfather had both worked at "the bomb plant" years before Frank came along.

After graduation Frank plans to emphasize teaching undergraduate students, but will continue to pursue his various research interests. Frank and Adele currently reside in Durham, North Carolina, with a collection of pedigreed poikilothermic pets, and Blue, the slobberdor.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

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